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Short Communication

First Report of DHA-1 Producing Enterobacter cloacae Complex Isolate in Bulgaria

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The aim of the present study was to reveal the characteristics of an *Enterobacter cloacae* complex isolate producing DHA-1 AmpC enzyme recovered from a patient hospitalized in St Marina Hospital, Varna.

Materials and methods: Susceptibility testing, conjugation experiments, isoelectric focusing, PCR and sequencing were carrying out.

Results: Of 176 *Enterobacter* spp. isolates only one isolate was positive for bla_{DHA}. The sequencing revealed the presence of bla_{DHA-1} and $bla_{CTX-M-3}$. The antimicrobial susceptibility testing showed higher resistance rates to almost all beta-lactams (ceftazidime, cefotaxime, cefepime, amoxicillinclavulanic acid, piperacillin/tazobactam), tobramycin, gentamycin, trimethoprim/sulphomethoxazole and quinolones (ciprofloxacin and levofloxacin). The isolate was susceptible to imipenem, meropenem and amikacin. The isoelectric focusing showed a band at pI 5.4 without ceftazidime and cefotaxime activity; a band at pI 7.8 with cefoxitin activity and another - with pI 8.4 with cefotaxime activity. Conjugation experiments were successful only for $bla_{CTX-M-3}$ carrying determinants.

Conclusions: To the best of our knowledge this is the first report of DHA-1 producing isolate in Bulgaria. The emergence of DHA-1 producing *E. cloacae* complex demonstrates the possibility for further dissemination of the gene encoding this enzyme. Infectious control measures are needed for the prevention of this phenomenon.

Key words:

Bulgaria, AmpC, DHA-1, *Enterobacter cloacae* complex

INTRODUCTION

Species from *Enterobacter cloacae* complex can cause a wide range of nosocomial infections including respiratory infections, bloodstream infections (BSIs), urinary tract and surgical site infections.[1,2] In recent years, the level of resistance towards many antibiotic groups, especially beta-lactams, has increased due to acquisition of extended-spectrum beta-lactamases (TEM-, SHV-, CTX-M-) or carbapenemases (KPC, OXA-48, NDM).[3] Additional challenges are the chromosomal class C beta-lactamases (AmpC) characteristic for *Enterobacter* spp.[4] The treatment of infections caused by these organisms with 3rd generation cephalosporines can cause an induction of the AmpC production, which, combined with ampD gene mutation, can result in stable high level production of these enzymes (the strains are known as "derepressed mutants").[4] In some cases the chromosomal AmpC gene has been mobilized on mobile elements and thus transmitted with them. Nowadays several main fam-

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ilies of plasmid-mediated AmpC have been identified – CMY, DHA, FOX, MIR, ACC.[4] The chromosomal AmpC producer could become the reservoir for plasmid-mediated enzymes, which could be easily disseminated.[4] In Bulgaria until now only CMY-4 and AAC-1 enzymes have been reported among Klebsiella pneumoniae isolates.[5,6] There are no reports on plasmid AmpC enzymes in *Enterobacter* spp. in our country. The aim of the present study was to reveal the characteristics of a DHA-1 AmpC producing *Enterobacter cloacae* complex isolate recovered from a patient hospitalized in Saint Marina Hospital – Varna.

MATERIALS AND METHODS

During a survey on ESBLs producing *Enterobacter* spp., performed in the hospital, *Enterobacter cloacae* complex isolate was recovered from the urine sample of a 47-year-old patient hospitalized in the Nephrology Ward on 21 July 2016. The patient suffered from chronic glomerulonephritis. Species level identification was done by Phoenix (Becton Dickinson, USA). Antimicrobial susceptibility testing was performed by the agar diffusion method. The results were interpreted according to the EUCAST guidelines, 2017. Conjugation experiments were carried out with rifampicin-resistant *Escherichia coli* K12:W3110 Rif R lac(-) strain, in solid cation-adjusted Mueller-Hinton agar as described previously.[7] β-lactamases were characterized by analytical isoelectric focusing (IEF) with the laboratory prepared polyacrylamide gel with Pharmalyte pH 3.5 -9.5 (Amersham-Pharmacia), as previously described.[7] β-lactamases bands were visualized by staining with a 500 mg/L solution of nitrocefin (BD Biosciences). Bioassay was performed to reveal the hydrolytic activity of the bands as described previously.[7] The growth of the indicator strain identified the band with hydrolytic activity. DNA from the clinical isolate and *Escherichia coli* transconjugant were used as a template in PCR amplification. PCR experiments for beta-lactamase group detection with primers specific for ESBL (SHV, CTX-M) and plasmid-borne AmpC genes (CMY, DHA, FOX, AAC, ACT/MIR) were performed as described previously.[7,8] The primers binding outside the genes were used for sequencing.[7,9] The PCR amplification products were purified and directly sequenced using an ABI 3130xl Genetic Analyzer (Applied Biosystems). The sequences were compared with the reported sequences from GenBank using the Basic Local Alignment Search Tool (BLAST) program.

RESULTS

The PCR experiments for different groups of AmpC enzymes (CMY, FOX, MOX, ACC) were negative for 175 third generation cephalosporin resistant isolates of *Enterobacter* spp. Only one isolate was positive in PCR for blaDHA, as well as for blaCTX-M. The sequencing revealed the presence of blaDHA-1 and blaCTX-M-3. The antimicrobial susceptibility of the isolate showed higher resistance rates to almost all beta-lactams (ceftazidime, cefotaxime, cefepime, amoxicillin-clavulanic acid, piperacillin/tazobactam), tobramycin, gentamicin, trimethoprim/sulphomethoxazole and quinolones (ciprofloxacin and levofloxacin). The isolate was susceptible to imipenem, meropenem and amikacin. The isoelectric focusing showed bands at: pI 5.4 without ceftazidime and cefotaxime activity (probably TEM-1 enzyme), pI 7.8 with cefoxitine activity (in this pI is focused DHA-1) and a band with pI 8.4 with cefotaxime activity (characteristic for CTX-M-3). The conjugation experiments were successful only for blaCTX-M-3 carrying determinants and the transconjugants were resistant to cefotaxime, tobramycin and gentamycin. The attempts to transfer blaDHA-1 were unsuccessful.

DISCUSSION

Laboratory detection of AmpC enzymes is important for the effective antimicrobial therapy. CMY and DHA are the most common groups of plasmid AmpC enzymes identified in *Enterobacter* spp.[1,10-18] To the best of our knowledge this is the first report of DHA-1 producing *E. cloacae* complex isolate in Bulgaria. The isolate demonstrated multidrug resistance profile. Only carbapenems and amikacin showed preserved activity. The multiple resistance was found to be associated with DHA-1 AmpC and CTX-M-3 ESBL. Both enzymes produced bands in isoelectric focusing which confirmed their production. DHA-1 is the first member of DHA group. The number of enzymes included in this group has increased among isolates from family *Enterobacteriaceae* and is of medical concern as their production leads to treatment failure.[4] DHA-1 enzyme was first detected in a *Salmonella enteritidis* isolate.[19] It was also the first plasmid-encoded β-lactamase found to be inducible and expressed in high levels.[4,19] DHA family enzymes have been commonly detected in *E. coli* and *K. pneumoniae*.[14] DHA-1 dominated in Asian countries (Korea), and was the most common type of AmpC β-lactamase in Korea (66.6%), Japan (69%) and China 96.7%.[16,17] There are few reports about DHA-1 positive *Enterobacter* spp. isolates.[12-15]

The emergence of DHA-1 producing isolates demonstrates the possibility for further dissemination of the gene encoding this enzyme. Infectious control measures are needed for the prevention of this phenomenon.

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Первое сообщение об изоляте Enterobacter cloacae Complex в Болгарии

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Цель: этого исследования состояла в том, чтобы выявить характеристики изолята Enterobacter cloacae complex , продуцирующего фермент DHA-1 AmpC, полученного от пациента, госпитализированного в больнице „Св. Марина", Варна.

Материалы и методы: Были проведены тесты на восприимчивость, эксперименты по конъюгации, изоэлектрическое фокусирование, ПЦР и секвенирование.

Результаты: Из 176 изолятов Enterobacter spp. только один изолят был положительным на bla_{DHA}. Секвенирование показало наличие bla_{DHA-1} и $bla_{CTX-M-3}$. Тестирование воспримчивости к антимикробным препаратам выявило более высокие показатели устойчивости почти ко всем бета-лактамам (ceftazidime, cefotaxime, cefepime, amoxicillinclavulanic acid, piperacillin/tazobactam), tobramycin, gentamycin, trimethoprim/sulphomethoxazole и хинолонам (ciprofloxacin and levofloxacin). Изолят был восприимчив к imipenem, meropenem и amikacin. Изоэлектрическое фокусирование показало полосу pI 5.4 без активности ceftazidime и cefotaxime; полосу pI 7,8 с активностью cefoxitin и другую pI 8,4 с активностью cefotaxime. Эксперименты по конъюгации были успешными только для детерминант, несущих $bla_{CTX-M-3}$.

Заключение: Насколько нам известно, это первое сообщение о DHA-1, продуцирующем изолят в Болгарии. Появление DHA-1-продуцирующего *E. cloacae* complex указывает на возможность дальнейшего распространения гена, кодирующего этот фермент. Применение мер инфекционного контроля необходимо для предотвращения этого явления.