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A novel streptomycin and spectinomycin resistance Gene cassette occurence in *E. cloacae* isolated from Zhenjiang

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Abstracts: The aadA genes, encoding resistance to streptomycin and spectinomycin, have been found as gene cassettes in different gram-negative and gram-positive bacterial species. The present report has revealed that the sequence of a new gene, aadA5, combining with the trimethoprim resistance gene dfr17 occured in a class 1integron. The integron was identified in a nosocomial pathogen Enterobacter cloacae isolates, which indicated that integrons are specialized genetic elements that were capable of capturing, integrating and mobilizing gene-cassette by site-specific recombination and integron was a dangerous factor to lead to bacterial resistance.

Keywords: E. Cloacae, Class 1 Integron, Resistance

In recent years, Enterobacter cloacae (E. cloacae) has become a well-recognized nosocomial pathogen. E. cloacae isolates from clinical specimens may be resistant to multiple antibiotics. For example, E. cloacae is intrinsically resistant to ampicillin and narrow-spectrum cephalosporins [1]. And a substantial proportion of multiresistant E. cloacae isolates carry integrons. Integrons are specialized genetic elements that was capable of capturing, integrating and mobilizing gene cassettes by site-specific recombination. Gene cassettes mainly encoded antibiotics genes. There are five classes integrons involved in bacterial resistance to date, While class 1 integrons are most frequently found in antibiotic-resistant gram negative bacteria [2]. The structure of the class 1 integron includes 5' and 3' conserved segments and a variable region [3].

The aadA genes which were first repoted by Hollingshead and Vapnek [4], are the only characterized genes that encode both streptomycin and spectinomycin resistance, and many of these genes are found as gene cassettes in class 1 integron [5]. The present report characterizes the nucleotide sequence and expression of a novel streptomycin and spectinomycin resistance gene located as a gene cassette. In a two-year period (2005–2006), 15 multiresistant E. cloacae strains were isolated in our laboratory, which carried a aadA5-containing integron. To the best of our knowledge, E. cloacae strains carrying aminoglycoside-3'-adenylyltransferase genes have not been reported before. Therefore, we undertook this study to analyse the structure of the aadA5-containing integron.

1. Materials and Methods

1.1. Bacterial Strains and Susceptibility Testing

The E. cloacae isolates were identified by the conventional method, and with the VITEK system card (bioMérieux, Hazelwood, MO, USA). Antibiotic-containing discs (Oxoid Ltd., , England) were used for routine antibiograms by disc diffusion assay. MICs of antimicrobial agents were determined by agar dilution in a Mueller–Hinton Media (Oxoid Ltd., Basingstoke, Hampshire, England) according to the guidelines of the NCCLS [6]. Escherichia coli ATCC 25922 was used as MIC reference strain.

1.2. E. Cloacae DNA Extraction

The E. cloacae DNA was extracted using the boiling lysated method [7]. The template was prepared by suspending a loopful of each isolate in 200mL of sterile water, followed by boiling for 10 min and centrifuging for 3 min.

1.3. Amplification and Sequencing of Class 1 Integrons

The gene cassette regions for the class 1 integrons were performed by PCR amplification with the following sets of primers: for the class 1 integron, 5′CS-F (5'-GGC ATC

CAA GCA GCA AG-3') and 3′CS-R (5'-AAG CAG ACT TGA CCT GAT-3') (8). PCRs were performed in volumes of 15μl under the following conditions: 1.5μl 10×PCR Buffer (Mg_2^+ plus), 0.2 mM of each deoxynucleoside triphosphate, 0.1 μM of each primer, and 1.5 units of Taq DNA or LA Taq DNA polymerase (Takara Biotechnology (Dalian) Co., Ltd.). for 10 min at 94°C; 35 cycles, with 1 cycle consisting of 45 seconds at 94°C, 1 to 2 min at 55 to 60°C, and 1 to 2 min at 72°C, and a final step of 10 min at 72°C. Sequencing reactions were performed by Shanghai GeneCore BioTechnologies Co.,Ltd. Database similarity searches for nucleotide and deduced amino acid sequences were carried out at the NCBI website (http://www.ncbi.nlm.nih.gov).

1.4. Nucleotide Sequence Accession Number

The nucleotide sequences of the aadA5 gene of E. cloacae isolate have been assigned to the GenBank nucleotide sequence database (GenBank accession no EF571855).

2. Results and Discussion

2.1. Properties of 15 E. Cloacae Strains

MIC testing revealed that E. cloacae isolates was resistant to most β -lactams, including ampicillin, ampicillin–sulbactam, piperacillin, piperacillin–tazobactam, cefalothin, cefoxitin, cefotaxime, ceftazidime, aztreonam, and E. cloacae isolates was high-level resistant to streptomycin and spectinomycin. MICs of aztreonam for the isolate was 64 mg/L. MICs of ampicillin, ampicillin–sulbactam, piperacillin, piperacillin–tazobactam, cefalothin, cefoxitin, cefotaxime and ceftazidime were>128 mg/L.

2.2. Sequence Analysis of the aadA5-Containing Integron

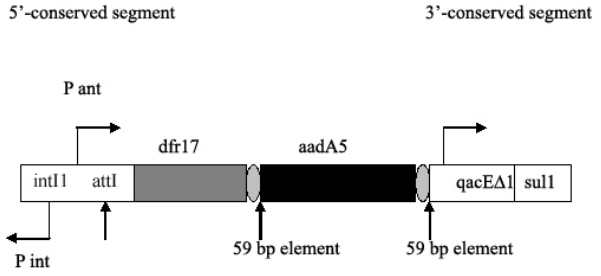

Fig 1. *Schematic map of the novel class 1 integron containing the aadA5 gene cassette from the E. cloacae isolate. Designations are as follows: intI1, integrase; attI, recombination site; dfr17, dihydrofolate reductase (trimethoprim resistance) gene; aadA5, streptomycin and spectinomycin resistance gene; qacEΔ1, quaternary ammonium compound resistance gene; sulI.*

PCR-based experiments showed that the aadA5 gene occurred 15 multiresistant E. cloacae strains and also found the 15 isolates had the identical amplicon. Sequence analysis of the 1664 bp amplicon revealed the structure of the class 1 integron, such as the 5′-CS element containing an IntI1 integrase gene with its own promotor region and the 3′-CS element containing qacEΔ1. The integron contained insert gene cassettes dfr17, aadA5, and two putative 59 base element. The aadA5 gene was located immediately downstream of the first putative 59 base element. The aadA5 gene cassettes had a 59 bp element, and the qacEΔ1 gene also had a core site (Fig. 1).

The aminoglycoside-3'-adenylyltransferase genes have spread among gram-negative bacilli, including Pseudomonas aeruginosa, Acinetobacter baumannii and Escherichia coli [8,9]. However, this enzyme was rarely found in E. cloacae. The presence of the mobile aadA5 gene cassette integron in E. cloacae suggests that the resistance can spread to other members of the family Enterobacteriaceae [10-12]. In the new class 1 integron in this study also contained a dihydrofolate reductase gene (dfr17) besides aadA5 cassette, suggesting that the gene responsible for the trimethoprim resistance.

The present study has characterized a novel streptomycin and spectinomycin resistance gene cassette found in a class 1 integron. The distribution of this gene is still to be investigated. The novel gene cassette is present in a class 1 integron, which is mobile. These factors enhance the mobiling possibilities for the aadA5 gene, and the conditions of transfer and the molecular epidemiology of this gene cassette will need further attention in the future.

3. Conclusion

The present work indicated that integrons are specialized genetic elements that were capable of capturing, integrating and mobilizing gene-cassette by site-specific recombination and integron was a dangerous factor to lead to bacterial resistance.

Acknowledgments

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