



letter

Molecular Identification of *MefE* and *AmpC* Resistance Genes in ATCC Bacteria

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Dear Editor,

Enterococci are gram-positive bacteria and the source of recurrent nosocomial infections with high levels of antibiotic resistance (1), of which we can name resistance to cephalosporins, aminoglycosides, monobactams, penicillinase resistance penicillins, and most importantly, vancomycin (2). Between 85% to 90% of the enterococci infections are caused by *Enterococcus faecium* (2), and macrolide-lincosamide-streptogramin is an antibiotic, which can be useful for treating enterococcal infections (1). M phenotype refers to a resistance mechanism to macrolides (such as erythromycin) and includes active-drug efflux pumps that are encoded by *mef* genes (3,4). For the first time, bacterial antibiotic efflux was reported in 1970 (3). The presence of *mef* genes have been reported in previous studies (6). In the current study, we reported *mefE* gene in *E. faecium* ATCC (American Type Culture Collection) 51559. *Acinetobacter baumannii* is a gram-negative bacterium that has turned into a great concern in the health care centers especially in intensive care units (4). The name *Acinetobacter* is originated from akinetos, a Greek word meaning non-motile (4). *A. baumannii* is a member of "ESKAPE" (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) group which consist of multi-drug resistant organisms (4). It is one of the important causes of nosocomial infections (5). Among these multi-drug resistance traits, chromosomal *AmpC*, a non-inducible cephalosporinase, was already reported in *A. baumannii* genome (4). In this study, we reported *AmpC* resistance gene in *A. baumannii* ATCC 19606. The mentioned ATCC bacteria, which were isolated from clinical

samples for the first time (6,7), were bought commercially. The Clinical and Laboratory Standards Institute (CLSI) guidelines were followed for the determination of resistance phenotypes (8). Suspensions of both *A. baumannii* ATCC 19606 and *E. faecium* ATCC 51559 were prepared with an opacity equivalent to 0.5 McFarland solution, and subsequently cultured on separate Mueller-Hinton agar plates. An ampicillin (10 µg) disc was used to check the phenotypic resistance in *A. baumannii* ATCC 19606 and an erythromycin (15 µg) disc for *E. faecium* ATCC 51559. The plates were then incubated for 24 hours at 35°C. *Staphylococcus aureus* ATCC 25923 was used for the quality control of both discs. Both *A. baumannii* ATCC 19606 and *E. faecium* ATCC 51559 exhibited resistance to the above-mentioned antibiotic discs as shown in Figure 1. In the next step, DNA was extracted using boiling method (9); a loopful of each bacterium was suspended in 1 mL of distilled water and boiled for 15 minutes. Then each microtube was centrifuged at 15000 g for 10 minutes. The DNA containing supernatants were used for PCR. The primers F: 5'-CAATATGGGCAGGGCAAG-3' and R: 5'-AAGCTGTTCCAATGCTACGG-3' were utilized for *MefE* gene detection (10), and the primers F: 5'-TAAACACCACATATGTTCCG-3' and R: 5'-ACTTACTTCAACTCGCGACG-3' for the *AmpC* gene detection (11). Both PCRs were carried out using 10 µL of the Master Mix Red (Amplicon, Denmark), 0.5 µL of each primer, and 3 µL of the respective DNA templates, and then brought to a total volume of 25 µL by adding deionized distilled water. Reaction cycles for *MefE* gene included an initial 7-minute denaturation at 95°C, followed by 44 cycles of: denaturation at 95°C

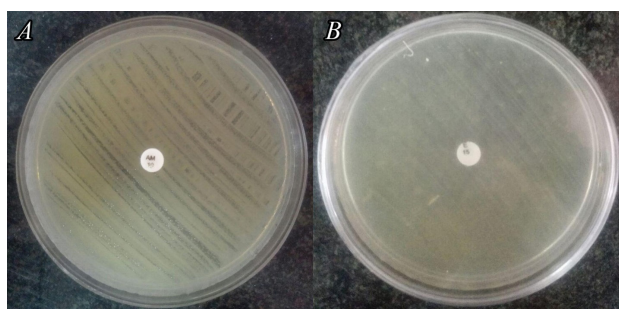


Figure 1. Detection of Phenotypic Resistance.

A: *A. baumannii* ATCC 19606 exhibits resistance phenotype to ampicillin (10 µg) disc.

B: *E. faecium* ATCC 51559 exhibits resistance phenotype to erythromycin (15 µg) disc.

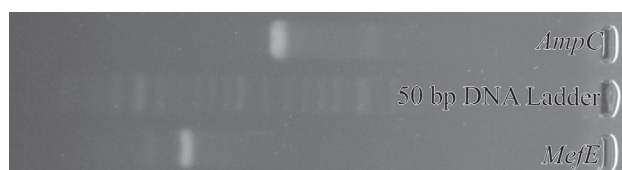


Figure 2. Expected Bands on 1.5% Agarose Gel Electrophoresis.

AmpC gene: 663 bp; *MefE* gene: 317 bp.

for 30 seconds, annealing at 65°C for 30 seconds, and extension at 72°C for 50 seconds, and final extension step at 72°C for 5 minutes. In the case of *AmpC* gene, the initial denaturation was at 95°C for 3 minutes, followed by 35 cycles of: 94°C for 1 minute, 56°C for 1 minute, 72°C for 2 minutes, and a final extension at 72°C for 10 minutes. A 1.5% agarose gel electrophoresis was used for the analysis of PCR products. The expected bands, 317 bp for *MefE* gene and 663 bp for *AmpC* gene, were observed (Figure 2). PCR products were sent to the Bioneer Company, South Korea, for sequencing. The results were confirmed by sequencing. Finally, sequences were registered on the GenBank. The accession numbers for *A. baumannii* and *E. faecium* are MF290419 and MF290421, respectively. Since the antibiotic resistance, especially multidrug resistance is a critical medical issue, detection and assessment of antibiotic resistance genes is of great importance.

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