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Paper Poster Session V

More and more third generation cephalosporin-resistent enteric bacteria everywhere?

Molecular characterization of genes encoding CTX-M-134, TEM-207 and TEM-212 detected among *Escherichia coli* clinical isolates from USA hospitals

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Objectives: To characterize three new beta-lactamase-encoding genes detected in *E. coli* clinical isolates collected in USA hospitals during the SENTRY Antimicrobial Surveillance Program.

Methods: Genes encoding new variants and its closest known beta-lactamase genes were amplified and cloned into a plasmid vector and transformed into a common *E. coli* background. Clinical isolates were submitted to conjugation and/or transformation. Genetic location of new genes was assessed by S1 nuclease and ICeuI followed by Southern blot and probe hybridization. All clinical isolates, transformants and transconjugants were susceptibility tested by reference broth microdilution methods. Incompatibility factors of plasmids carrying new genes was determined by a multiplex PCR method. Primer walking was used to reveal the genetic environment of the new genes. MLST was also performed.

Results: $bla_{\text{CTX-M-134}}$ was detected in an *E. coli* clinical strain collected on 5/4/2011 from a bile specimen of a 70 y/o female patient hospitalized in the ICU after surgery at a hospital in Lexington, KY. $bla_{\text{TEM-207}}$ was observed in an *E. coli* isolate collected on 4/25/2012 in Gainsville, FL from a sputum specimen of a 60 y/o male patient. Two *E. coli* isolates carrying $bla_{\text{TEM-212}}$ were detected in a hospital located at Sun City, AZ. The isolates were collected from different ICU patients, both males of 87 and 19 y/o in a three month interval. All isolates had modestly elevated MIC values (1-8 mg/L) for broad-spectrum cephalosporins and TEM-producing isolates were also resistant to piperacillin/tazobactam (MIC, >64 mg/L). All isolates carried no other beta-lactamase encoding genes. When expressed in an *E. coli* background, CTX-M-134 encoded resistance to ceftazidime, cefepime and ceftriaxone (MIC, ≥16 mg/L) and MIC values were comparable to those for CTX-M-14 (closest variant; 99.7% similarity) in the same background. TEM-207 was an ESBL gene and TEM-212 was an inhibitor resistant narrow-spectrum enzyme. $bla_{\text{CTX-M-134}}$ was located in a 66-Kb self-conjugative FIA/FIB/FIC incompatibility plasmid and this gene was flanked upstream by IS*Ecp1* and downstream by IS903D. The gene encoding TEM-207 was located in a transposon element (*tnpR*-$bla_{\text{TEM-207}}$-Ins*A*-*yjcA* hypothetical protein encoded) carried by a 120-Kb plasmid that was transformed (but not conjugated) to an *E. coli* host. In both clinical isolates $bla_{\text{TEM-212}}$ was embedded in a 148-Kb A/C incompatibility type self-conjugative plasmid and this gene was flanked upstream by *tnpR* and downstream by IS*26*. Isolates carrying $bla_{\text{CTX-M-134}}$ and $bla_{\text{TEM-207}}$ belonged to ST131.

Conclusions: We report three new beta-lactamase genes all detected in *E. coli* clinical isolates collected in USA hospitals. Two of these clinical isolates belonged to ST131, a strain with known virulence factors that cause of severe infections and the other gene was observed in isolates collected from the same hospital within a short time interval, all being potential causes of concerns for microbiologists and infectious diseases practitioners.