AMENDE ABSTRACT

The objective was to evaluate 8-lactam resistance mechanisms in P. aeruginosa from Chinese hospitals and to identify the prevalence of intrinsic resistance mechanisms among Chinese PSA. All 12 isolates displaying ceftazidime and/or carbapenem resistance were further evaluated. Results: Ten isolates possessed MLS-BLs (MLS-BLs; M-III, M-III-V, M-IV); 2 isolates possessed MLS-A. Two isolates from the same hospital had the same PFGE and ST profile and two PSA from different hospitals had the same ST profile despite the different PFGE patterns.

 MATERIALS AND METHODS

Bacterial strains: A total of 12 P. aeruginosa isolates were collected from different hospitals during susceptibility testing performed by the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI, 2013). Endogenous reference gene (rpsL) transcription levels of the chromosomally encoded AmpC (ampC), MexCD-OprJ (mexC) and MexEF-OprN (mexE) were determined by real-time PCR. RT-PCR was performed using DNA-free RNA preparations. Total RNA was extracted from mid-log phase bacterial cultures (cell density of 0.5–1.5×10⁷ cfu/mL) using a QiaShredder and QIAshuttle RNAPrep Kit (Qiagen) and residual DNA was eliminated with RNase-free DNase for further processing. Genomic DNA was prepared in agarose blocks and digested with SpeI DNA polymerase by multiplex PCR. Genomic DNA from each isolate was amplified in at least two multiplex polymerase chain reactions (PCR). PCR reactions for the detection of blaOXA-30, blaOXA-48, and blale were performed as previously described. All strains were identified among 12 isolates displaying 11 unique PFGE types. Two PSA from the same hospital had the same PFGE and ST profile and two PSA from different hospitals had the same ST profile despite the different PFGE patterns.

RESULTS

Conclusions: Resistance mechanisms were very diverse among 12 ceftazidime- and carbapenem-nonsusceptible P. aeruginosa from Chinese hospitals. Four isolates harboured acquired 8-lactamases, including MLS-BLs in most cases, hyperexpression of AmpC or MexAB-OprM. Decreased expression of oprD was present.

Phenotypic tests of efflux pump and AmpC inhibitors did not correlate with the genotypes established by quantitative RT-PCR, but a much better correlation was noted when both inhibitors were tested in combination.

SELECTED REFERENCES


INTRODUCTION

Carbapenem resistance among Pseudomonas aeruginosa clinical isolates has increased worldwide and this problem has become a global public health concern. The limited therapeutic options available to treat infections caused by multi-resistant P. aeruginosa highlight the need for continued research in this area. Acquired carbapenem resistance in P. aeruginosa is often associated with AmpC β-lactamases and efflux pumps. In this study, we evaluated intrinsic and extrinsic resistance mechanisms among 12 isolates from Chinese hospitals including ceftazidime and imipenem resistant isolates collected during 2011 in six Chinese hospitals.

Molecular typing and susceptibility patterns of ceftazidime and/or carbapenem resistant isolates from Chinese hospitals. Testing using clavulanic acid is not displayed due to no significant differences observed.

RESULTS

Eleven isolates from Chinese hospitals during 2011 showing variable resistance to carbapenems and 6 isolates were further evaluated. Expression of AmpC and MexAB-OprM hyperexpression are most common mechanisms among genetically diverse PSA. The IMP-9 producing strain also harboured this MßL alone or with PER-1/-5 with or without imipenem resistance. Additionally, inducible AmpC can be upregulated by clavulanic acid. In this work, we performed the same PFGE and ST analysis to identify the prevalence of intrinsic resistance mechanisms among Chinese PSA, but also highlights that multiple factors might contribute to elevated PSA resistance rates in this country.