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**Resistance surveillance & epidemiology: Gram-negatives**

# Acinetobacter baumannii isolated from respiratory samples as part of the MagicBullet clinical trial: molecular epidemiology and susceptibility to carbapenems, tigecycline and colistin

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**Background:** MagicBullet is a multi-center European collaborative research project investigating two empirical antimicrobial regimens, i.e. colistin vs. meropenem, both combined with levofloxacin, to treat ventilator-associated pneumonia (VAP). The objective of our study was to investigate the molecular epidemiology, carbapenem, colistin and tigecycline susceptibility, and carbapenem-resistance determinants of *Acinetobacter baumannii* isolated from respiratory tract samples of VAP patients enrolled in MagicBullet.

**Material/methods:** The first *A. baumannii* isolates cultured from respiratory tract samples were collected from VAP patients from 33 hospitals in Greece, Italy and Spain from 05/2012 to 10/2015. Species identification was performed by MALDI-TOF and *gyrB* multiplex PCR. Susceptibility testing was performed by Etest. Carbapenem resistance determinants were identified by PCR and sequencing. Molecular epidemiology was investigated using rep-PCR (DiversiLab), with a similarity of ≥ 98.6% interpreted as identical. International clones (IC) were identified with a similarity of ≥ 95% compared to our reference database.

**Results:** Sixty-three *A. baumannii* isolates were collected from respiratory tract samples from patients hospitalized with VAP in 15 hospitals. The majority of isolates were resistant to the carbapenems with MICs of > 32 mg/L (Table 1). Only two isolates were carbapenem-susceptible with imipenem and meropenem MICs of 0.25 mg/L and 2 mg/L, respectively. Carbapenem resistance was always associated with acquired carbapenemases: OXA-23, OXA-40 and OXA-58 were identified in 50, 3 and 7 isolates, respectively. Furthermore, one isolate harboured both OXA-23 and OXA-58. In all instances, OXA-23 and OXA-58 genes were associated with insertion elements IS*Aba1* and IS*Aba3*, respectively. Almost 62% of isolates were resistant to tigecycline with MICs > 2 mg/L, but 71% remained susceptible to colistin with MICs ≤ 2 mg/L (Table 1). The majority of isolates (n=55) represented IC2, with four subtypes identified (C (n=45), D (n=1), E (n=6), F (n=3)). Subtype C comprised isolates originating from 11 hospitals enrolled in MagicBullet. IC1 was represented by five

isolates (G (n=4), H (n=1)) from three Greek hospitals, while the remaining three isolates (A (n=2), B (n=1)) originating from two hospitals in Spain and Italy, respectively, were unclustered.

**Conclusions:** Carbapenem resistance was almost universal in *A. baumannii* cultured from respiratory samples of VAP patients. Alarmingly, more than 60% of the isolates showed elevated MICs to tigecycline. This highlights the difficulty in empirical treatment of *A. baumannii* VAP. Although the majority of isolates remained susceptible to colistin, resistance to this compound of 29% is alarming. Rep-PCR confirms that IC2 is currently the predominant lineage and suggests the presence of an epidemic *A. baumannii* clone that has spread within Greece, Italy and Spain.

Table 1: Distribution of resistance and susceptibility to the tested antibiotics in *A. baumannii* isolates.

| | % susceptible | % resistant |
|---|---|---|
| Imipenem | 3.2 | 96.8 |
| Meropenem | 3.2 | 96.8 |
| Tigecycline* | 38.1 | 61.9 |
| Colistin | 71.4 | 28.6 |

*EUCAST breakpoint Enterobacteriaceae