

Characterization of colistin-resistant *A. baumannii* isolated in Intensive Care Unit of an Italian Hospital

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SUMMARY

We report the characterization of an *Acinetobacter baumannii* resistant to colistin isolated from a patient treated with colistin for 22 days.

The identification and initial susceptibility testing of the strain was performed at ASL3 Imperiese with the Vitek-2 automated system and then the strain was re-identified at the Sezione di Microbiologia with APINE. *In vitro* activity of antimicrobial agents was determined by the microdilution methods. The detection of the beta-lactamase gene was performed by PCR and for the analysis of LPS were sequenced the genes of the PmrABC system.

We compared the PFGE profile of the colistin-resistant *A. baumannii* with 5 *A. baumannii* colistin-susceptible strains isolated in the same Hospital during 2010.

The strain was resistant to all antimicrobial agents tested, except to tygecyclin. The PFGE shown that the *A. baumannii* colistin-resistant was closely related to the colistin-susceptible strains.

The sequencing of the PmrABC system showed that the strain had a single mutation in the PmrB component. Colistin-resistant strain have recently been found in several Gram-negative bacteria such as *A. baumannii*, *K. pneumoniae* and *P. aeruginosa*.

This clinical case confirms that resistance to colistin in *A. baumannii* can be selected *in vivo* following the use of colistin in therapy.

INTRODUCTION

Acinetobacter baumannii is an important pathogen in Intensive Care Unit (ICU) patients. In nosocomial *A. baumannii* isolates resistance to extended-spectrum beta-lactams, carbapenems, aminoglycosides and fluoroquinolones are widespread. Colistin is the last resort for treatment of MDR *A. baumannii*, unfortunately, however, resistance to colistin has been reported all over the world. The mechanism of resistance is not yet clear, but some study confirm that it might be related to a loss of LPS (12, 13, 7) or/and the PmrAB two component system (1, 14).

We report the characterization of a colistin-resistant *A. baumannii* isolated from a 85 years old man after colistin therapy.

We compared the PFGE profile of the colistin-resistant *A. baumannii* with those of other *A. baumannii* strains isolated in this geographic area.

Bacterial strains and patients

The patient was transferred on 08/31/11 to ASL3 Imperiese Hospital from the ICU of Santa Corona Hospital where he was hospitalized following

chest trauma with multiple injuries. At the admission to ASL3 the patient was already tracheostomized.

On day 1, a colistin-susceptible *A. baumannii* isolate (310811) was recovered from broncho-lavage, the patient was treated with voriconazole and piperacillin/tazobactam. On day 13 a throat swab was positive to the colistin-susceptible *A. baumannii*, while broncho-lavage was negative. The treatment was switched to meropenem, voriconazole and colistin. On day 20, a throat swab was positive to the colistin-susceptible *A. baumannii*, while broncho-lavage was negative. The treatment was switched to meropenem, ciprofloxacin and colistin. On day 35 a colistin-resistant *A. baumannii* isolate (051011) was recovered from broncho-lavage. A colistin resistant *A. baumannii* was also present in broncho-lavage by day 43 and in throat swabs and in broncho-lavage by day 50. On day 53 the patient died.

A. baumannii (310811), *A. baumannii* (051011) and 10 colistin-susceptible *A. baumannii* isolated at ASL3 Imperiese Hospital in 2010 from different clinical sample (blood, broncho-lavage, bron-

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cho-aspirate, catheter tip and oral swab) were characterized by pulsed-field gel electrophoresis.

METHODS

The identification and initial susceptibility testing of *A. baumannii* isolate was performed with the Vitek-2 automated system (bioMérieux, France). The strain was sent to Sezione di Microbiologia, University of Genoa for further investigation.

The strain was re-identified with API NE (bioMérieux, France). *In vitro* activity of ampicillin, amoxicillin/clavulanate, piperacillin/tazobactam, ceftazidime, imipenem, gentamicin, ciprofloxacin, trimethoprim-sulfamethoxazole and tigecycline was determined by the broth microdilution methods and interpreted according to the Clinical Laboratory Standard Institute (CLSI) (5). In addition, MIC of colistin was determined by the agar dilution (11) and interpreted using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (6).

The detection of beta-lactamase gene was performed by PCR, using previously condition and primers for *blaOXA51*, *blaOXA23*, *blaOXA24* and *blaOXA58* (18).

The genetic relatedness of *A. baumannii* colistin-resistant (051011), susceptible isolated from BAL (310811) and 5 *A. baumannii* isolates in 2010, was determined by molecular typing using pulsed-field gel electrophoresis (PFGE) as follows: genomic DNA, extracted and digested with *ApaI* (New England Biolabs Inc., MA, USA) as previously described (16), was separated by PFGE using CHEF DRII device (Bio-rad, Hercules, CA, USA). Running conditions were as follows: 27h at 11.3°C at voltage of 5.4V, ramped with an initial forward time of 5s and final forward time of 40s. After electrophoresis, gel was stained with ethidium bromide (1µg/ml) and visualized on an ultraviolet transilluminator.

The banding patterns were interpreted visually following published guidelines (17).

Primer and conditions previously described (3) were used for sequencing the PmrAB two components system. The amplicons were purified using High Pure PCR Cleanup MicroKit (Roche) and sequenced as previously described (4).

RESULTS

The isolate showed a multidrug-resistant phenotype, being susceptible only to tigecycline. MIC to colistin was 16µg/ml.

The strain was positive for *blaOXA51* and *blaOXA23* by PCR.

The PFGE of *ApaI* digested genomic DNA of *A. baumannii* colistin-resistant and *A. baumannii* colistin-susceptible and of 5 *A. baumannii* colis-

tine susceptible isolate from the same ward in 2010 revealed that *A. baumannii* colistin-resistant and colistin-susceptible were closely related, this clone has been found circulating in this hospital since 2010.

The colistin-resistant isolate (051011) had a single aminoacid change in PmrB (Pro233Ser). No differences were found in the *pmrA* or *pmrC* gene.

DISCUSSION

Colistin-resistant isolates have recently been identified in several Gram-negative bacterial species, such as *A. baumannii*, *K. pneumoniae* and *P. aeruginosa* (2, 8, 9). In 2011 Kempf-Rolain (15) and López-Rojas (10) reported in France and in Spain, respectively, two cases of patients with *A. baumannii* becoming resistant to colistin after colistin therapy. Our case report confirms that colistin-resistant *A. baumannii* strain may be selected *in vivo* by the use of colistin.

The results of PFGE indicated a unique epidemic clone circulating in ASL3 Imperiese Hospital, where all strains, including the *A. baumannii* colistin-resistant were closely related. The presence of a unique clone persistently occurring in an ICU is also connected to the capability of the microorganism to survive for long periods in the hospital environment, even in dry conditions.

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