Emergence of colistin resistance during treatment of recurrent pneumonia caused by carbapenemase producing Klebsiella pneumoniae

Emergence of colistin resistance during treatment of recurrent pneumonia caused by carbapenemase producing *Klebsiella pneumoniae*

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Abstract

A 60 y/o woman received meropenem/colistin treatment for bilateral pneumonia caused by a ST15 carbapenemase producing *Klebsiella pneumoniae*. The patient recovered but re-

infection with the same (ST15), but now colistin-resistant *K. pneumoniae*, occurred. The molecular mechanism of the emerged colistin resistance was identified as *mgrB* gene modification by insertion element (IS) IS903B.

Colistin (polymyxin E) is a last-resort antimicrobial agent reserved for treating infections with multi-drug resistant Gram-negative bacilli. However, due to the increased consumption of the drug, reports on colistin resistance have increased during the past years, especially when used in monotherapy (Osei Sekyere et al. 2016). Therefore, its use in double or triple combinations is advised (Osei Sekyere et al. 2016). The main mechanism for colistin resistance is explained by modification of the lipid A moiety of lipopolysaccharides, the primary target molecules of polymyxins (Baron et al. 2016). Colistin resistance can be mediated by chromosomal mutations and by the more recently detected plasmid-borne *mcr* genes (Sun et al. 2018). We describe the *in vivo* emergence of colistin resistance in *Klebsiella pneumoniae* after a 2-week course of colistin in combination with meropenem as treatment for pneumonia.

A 60-year old female patient was transferred to the intensive care unit of the Antwerp University Hospital in January 2017 (Figure 1). The patient's comorbidities included diabetes mellitus type 2 requiring insulin therapy, kidney transplantation in 2010 for which she received immunosuppression with mycophenolate mofetil and sirolimus, hypothyroidism, asthma and atrial fibrillation. She had been hospitalized for a month in a peripheral hospital for a community acquired bilateral pneumonia with respiratory insufficiency requiring intubation. Despite antibiotic treatment and tracheotomy, weaning problems persisted, necessitating transferal to our tertiary care center. At the time of transfer, the patient was treated with meropenem and teicoplanin since ESBL producing *Klebsiella pneumoniae* and *Enterococcus faecalis* had been isolated from the patient's blood cultures and/or bronchial aspirate. In the first endotracheal aspirate (ETA) culture in our hospital only

Stenotrophomonas maltophilia was present and blood cultures remained negative. Therefore, antibiotic treatment was discontinued. Because of abundant production of viscous purulent secretions, compatible with active lower respiratory tract infection, treatment with trimethoprim/sulfamethoxazole was initiated. Mid-January, the patient developed type 2 respiratory insufficiency with renewed need for mechanical respiratory support. A new ETA culture revealed the presence of *Enterobacter cloacae complex* and *Pseudomonas aeruginosa* for which meropenem treatment (1g IV q12h, adjusted dosing for a creatinine clearance of less than 30 ml/min) was restarted. The bronchial aspirate culture a week later demonstrated growth of *Enterobacter cloacae complex* (sensitive to amikacin, meropenem MIC 0.5 mg/L and colistin 0.5 mg/L) and *K. pneumoniae* (sensitive to amikacin, meropenem MIC 0.25 mg/L and colistin 1 mg/L) (designated isolate 1). Both strains were resistant to the other tested antibiotics including amoxicillin-clavulanic acid, piperacillin-tazobactam, temocillin, 1^{st}- 4^{th} generation cephalosporins, aztreonam, ciprofloxacin and trimethoprim/sulfamethoxazole. Disk diffusion (Rosco, International Medical Products) was used for susceptibility testing, except for meropenem (E-test, BioMérieux) and colistin (broth microdilution with sensititre (ThermoFisher Diagnostics), shown recently to be the most reliable commercial colistin broth microdilution method (Jayol et al. 2018)). Interpretation of antibiotic susceptibility was done according to EUCAST (EUCAST 2017). Both strains were identified as carbapenemase-producing Enterobacteriaceae (CPE) by an in-house phenotypic screening assay for rapid detection of carbapenemase production (Nordmann et al. 2012), using the screening criteria for carbapenemase production of the EUCAST guidelines for detection of resistance mechanisms (EUCAST 2013). Due to the presence of the 2 CPEs, colistin (6 MIU IV loading dose followed by 2 MIU IV q8h) was added to the meropenem treatment. Antibiotic therapy was discontinued after 14 days of colistin and 21 days of meropenem treatment as the patient's condition was stabilized and only commensal flora was

isolated from ETA. The patient was transferred to the pneumology ward mid-February. Due to emergence of fever and CRP elevation, a new bronchial aspirate was taken on March 7th which showed growth of a carbapenemase producing *Klebsiella pneumoniae*, but now resistant to colistin (MIC 4 mg/L). The strain was still sensitive to tigecycline (MIC 0.5 mg/L by E-test, BioMérieux) and meropenem, but the MIC-value for meropenem had increased to 2 mg/L. The infection was successfully treated with meropenem (2g IV q12h) and tigecycline (50 mg IV q12h). However, another pneumonia episode with a colistin-resistant (MIC 16 g/L) *Klebsiella pneumoniae* (designated isolate 2) followed in May requiring intubation and treatment with meropenem, amikacin and tobramycin aerosol for 3 weeks. The patient slowly recovered and was discharged from the pneumology ward July 25th.

The two *K. pneumoniae* isolates (isolate 1 = pre-colistin treatment and isolate 2 = post- colistin treatment) were analyzed by whole genome sequencing using Nextera XT, 2 x 250 bp, MiSeq, Illumina (Illumina Inc., USA) to understand the resistance mechanism underlying the observed phenotypic change in colistin susceptibility. Comparative genome analysis revealed that both *K. pneumoniae* strains belong to the same ST type, ST15, which is a type previously reported as involved in emergence of colistin resistance (Mammina et al. 2012). Both our strains showed a similar accessory genome profile (plasmid, virulence genes and resistance genes like $bla_{\text{CTX-M-15}}$ and $bla_{\text{SHV-28}}$ coding for extended spectrum beta-lactamases and $bla_{\text{OXA-48}}$ coding for a carbapenemase) and only 12 core genome SNP differences were identified between the 2 isolates, suggesting that both strains were genotypically the same (relatedness SNP threshold ≤ 18 for *K. pneumoniae*) (Schürch et al. 2018). Other than the SNPs, the only genetic difference identified was a *mgrB* gene modification by the insertion of IS903B (belonging to IS5 super family) in the intergenic region between the promoter and start of the gene (Figure 2). Inactivation of *mgrB* appears to be the most prevalent colistin resistance mechanism in *K. pneumoniae* and it leads to

activation of PhoPQ which upregulates *arnBCADTEF* that synthesizes 4-amino-4-deoxy-*L*-arabinose and transfers it to lipid A. Also, among *mgrB* modifications, IS truncation is most commonly observed, followed by nucleotide deletions, mis- or non-sense mutations and loss of the *mgrB* gene (Baron et al. 2016). Considering the genetic and phenotypic profile similarities between the 2 *K. pneumoniae*, we hypothesize that the 14-day colistin treatment triggered the transposition event that led to the modification of the *mgrB* gene in isolate 2. Treatment with colistin and the duration of colistin therapy have been proven to be major risk factors for the emergence of colistin resistance (Kontopidou et al. 2011). However, the colistin treatment duration in our case is rather short compared to a median treatment duration of 20 days that was shown to be a risk factor for colonization with colistin-resistant *K. pneumoniae* (Kontopidou et al. 2011).

In conclusion, this case report illustrates that even when colistin is used in bitherapy, emergence of resistance cannot be prevented and that infection with multi-drug resistant isolates is a major burden on individual patient management.

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Figure 1: Timeline of clinical events, laboratory findings and antibiotics administered during the hospital course. Abbreviations: ETA, endotracheal aspirate.

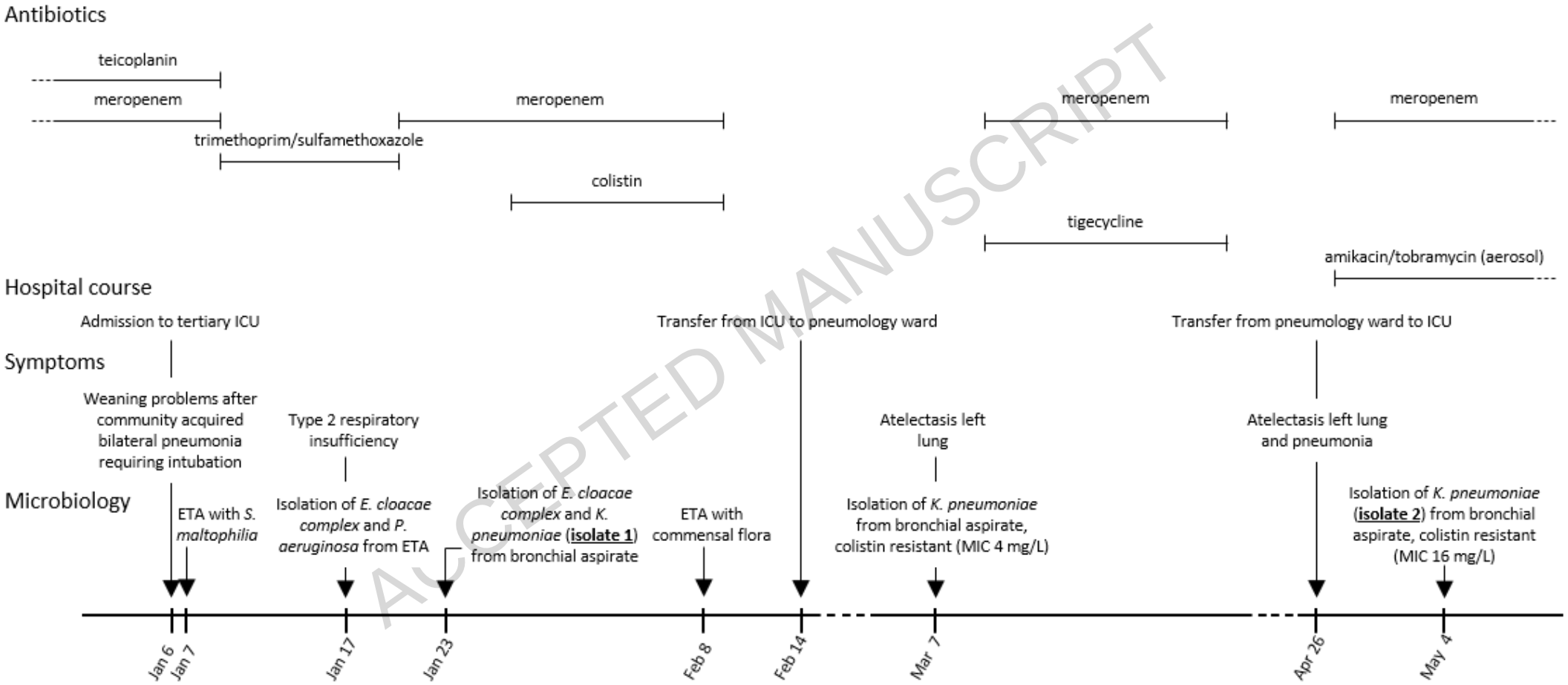

Figure 2: Comparative genome analysis between the colistin sensitive (isolate 1) and colistin resistant *K. pneumoniae* (isolate 2): IS903B (IS5 family) is transposed into the intergenic region between the promotor and start codon of the *mgrB* gene presumably under colistin pressure.