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Case Report

Therapeutic strategy for pandrug-resistant *Klebsiella pneumoniae* severe infections: short-course treatment with colistin increases the in vivo and in vitro activity of double carbapenem regimen

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SUMMARY

Infections due to carbapenemase-producing *Klebsiella pneumoniae* represent an emerging threat due to the high mortality rate and lack of valid antimicrobial combinations, especially when the strain is colistin-resistant. We report a case of bloodstream infection due to pandrug-resistant *K. pneumoniae* treated successfully with an innovative regimen comprising a combination of colistin plus double carbapenem, along with an in vitro analysis showing the synergistic and bactericidal effect.

1. Introduction

Infections due to carbapenemase-producing *K. pneumoniae* (CP-Kp) have been recognized as an emerging challenge worldwide as they are associated with a high mortality rate, especially when the strain is colistin-resistant.[1] Preliminary data suggest a role for unconventional antibiotic combinations against colistin-resistant CP-Kp, including colistin-based combinations.[2] However, the role of colistin in the setting of colistin-resistant strains is still debated. Among innovative approaches, the use of a double-carbapenem regimen has been proposed as a valid therapeutic option in severe infections due to CP-Kp.[3]

The present report describes a case of bloodstream infection due to pandrug-resistant (PDR) *K. pneumoniae* treated successfully with an innovative regimen comprising a combination of colistin plus double carbapenem. In vitro analysis of this regimen showed the synergistic and bactericidal effect.

2. Case report

A 75-year-old woman with a history of recent hip joint placement was admitted to the Orthopaedic Department of Sapienza University of Rome because of a delayed prosthetic joint infection. She underwent a hip replacement and antibiotic-loaded cement was placed. Intraoperative tissue and sonication fluid cultures grew *Staphylococcus hominis* resistant to methicillin and susceptible to rifampicin, fluoroquinolones, glycopeptides, and daptomycin. Therapy with rifampicin 600 mg/day and daptomycin 8 mg/kg/day was started and the patient was transferred to the Infectious Diseases Department. On admission, the patient was afebrile. Her erythrocyte sedimentation rate (ESR) was 87 mm/h and C-reactive protein (CRP) 9.63 mg/dl. A urinary catheter and central venous catheter (CVC) were placed. During therapy with intravenous rifampicin and daptomycin, normalization of the ESR and CRP was observed. However, after 15 days of hospitalization she developed a fever (body temperature 38.5 °C), hypotension, and tachycardia. The white blood cell count was 4.7×10^9/l, ESR 60 mm/h, and CRP 9.01 mg/dl. A chest X-ray was negative and the CVC was removed. Urine (10^5 CFU/ml) and blood cultures ($n = 3$) were positive for PDR *K. pneumoniae*; CVC cultures grew PDR *K. pneumoniae* and *Acinetobacter baumannii* resistant to carbapenems

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and sensitive to colistin, with a semiquantitative culture >15 CFU/ml. Thus, rifampicin was stopped and intravenous therapy with ertapenem 1 g/day and meropenem 2 g every 8 h was started. The following day, intravenous colistin was added (loading dose 6 000 000 IU, then 4 500 000 IU every 12 h). After 48 h of therapy, the patient remained febrile and blood cultures grew PDR *K. pneumoniae*. After 96 h she became afebrile. Laboratory analyses showed a reduction of the ESR and CRP. Blood and urine cultures did not grow any organism. After 7 days of therapy, colistin was stopped because of visual hallucinations, whereas the double carbapenem regimen was continued for an additional 14 days, in the absence of adverse events. The patient was discharged in good condition; she was afebrile with an ESR of 31 mm/h and CRP of 0.9 mg/dl. She was then transferred to the Orthopaedic Department for a new hip prosthesis replacement.

The VITEK-2 system was used for bacterial identification and antimicrobial susceptibility testing of the *K. pneumoniae*. In addition, minimal inhibitory concentrations (MICs) of ertapenem (ERT), meropenem (MEM), and colistin (COL) were determined by broth macrodilution method (BMD) in accordance with the Clinical and Laboratory Standards Institute guidelines.[4] For COL testing, a polysorbate 80 (Sigma-Aldrich) final concentration of 0.002% was used in order to avoid adherence of the drug to the tube wells. Carbapenemase production was confirmed phenotypically by double disk synergy testing.[5] Furthermore, the activity of MEM, ERT, and COL, alone and in combination, was investigated by time–kill studies using an initial inoculum of approximately 5×10^5 CFU/ml at the following concentrations: 1 × MIC ERT, 1 × MIC MEM, 1 × MIC COL, 0.5 × MIC ERT + 0.5 × MIC MEM, 0.5 × MIC ERT + 0.5 × MIC MEM + 0.5 × MIC COL, 1 × MIC ERT + 1 × MIC MEM, and 1 × MIC ERT + 1 × MIC MEM + 1 × MIC COL.

MICs of ERT, MEM, and COL, against the PDR *K. pneumoniae* were 128, 256, and 32 μg/ml, respectively. In addition, MICs of gentamicin and tigecycline were 8 and 4 μg/ml, respectively. Phenotypic analyses showed that the isolate was a *K. pneumoniae* carbapenemase (KPC) producer.

Despite an initial reduction in log CFU/ml, significant regrowth at 24 h was observed for ERT or MEM alone, whereas COL alone did not lead to a reduction in the bacterial count at any point in time (Figure 1).

When the double carbapenem combination was tested at a concentration of MEM 0.5 × MIC + ERT 0.5 × MIC, bactericidal activity was achieved at 4, 6, and 8 h; however, regrowth was observed at 24 h. In contrast, the bactericidal activity was maintained for up to 24 h at concentrations of MEM 1 × MIC + ERT 1 × MIC.

Of note, the combination of ERT plus MEM plus COL showed synergistic and bactericidal activity at 8 h, with the absence of bacterial growth, which was maintained for up to 24 h at both concentrations of ERT 0.5 × MIC + MEM 0.5 × MIC + COL 0.5 × MIC and ERT 1 × MIC + MEM 1 × MIC + COL 1 × MIC (Figure 1).

3. Discussion

Carbapenem-resistant *Enterobacteriaceae*, including *K. pneumoniae* and *Escherichia coli*, show high levels of resistance to carbapenems and other antimicrobial classes, with increasing reports of colistin resistance.[1] Therefore, in the absence of available antimicrobials, new therapeutic approaches against these emergent organisms are needed. Recently, the double carbapenem regimen has been proposed as a valid therapeutic option in severe infections due to PDR *K. pneumoniae*.[3] Nowadays, the conventional reporting of *K. pneumoniae* as resistant to all antimicrobials appears not to be sufficiently informative for the clinician; thus the performance of synergy testing in cases of infection due to PDR strains might be useful in selecting the best antimicrobial combination.

The case presented shows how the combination of colistin with ertapenem plus meropenem was effective and synergistic against a PDR *K. pneumoniae* causing bloodstream infection, even in the presence of high MIC values. The rationale for the use of colistin together with the double carbapenem regimen resides in the disruption caused to the outer bacterial cellular membrane by colistin allowing the other drugs to reach adequate intracellular concentrations. Moreover, given the high affinity for carbapenemases, ertapenem binds to the hydrolytic enzymes, acting as a suicide inhibitor and allowing the other carbapenem to exert bactericidal activity. The synergistic effect of this combination has been observed even in the setting of high carbapenem resistance.[3]

In the killing curves, carbapenems alone had initial bactericidal activity, but this was followed by significant regrowth at 24 h. As the MICs of both carbapenems were high, we could hypothesize that the initial but temporary activity of these drugs alone was due

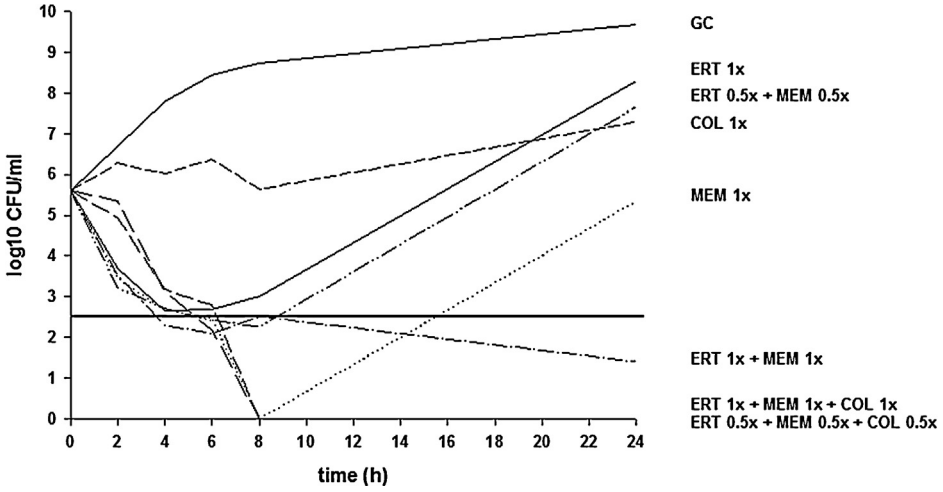

Figure 1. Time–kill studies for ertapenem, meropenem, colistin, ertapenem plus meropenem, and ertapenem plus meropenem plus colistin against pandrug-resistant *Klebsiella pneumoniae* isolated from a patient with a bloodstream infection. The horizontal line represents a reduction of 3 $\log_{10}$ CFU/ml compared with the initial bacterial count. GC, growth control; MEM, meropenem; ETP, ertapenem; COL: colistin.

to the high concentrations used in the in vitro experiments. In contrast, the combination of colistin with ertapenem plus meropenem showed rapid bactericidal activity, even at sub-inhibitory concentrations. Therefore, given the potent in vitro effect and the good clinical outcome of the patient even after colistin discontinuation, we suggest that colistin might be useful as an initial therapeutic add-on against PDR organisms, rapidly decreasing the bacterial amount and limiting drug toxicity.

In fact, our patient was treated with colistin plus double carbapenem for 7 days and colistin was then stopped because of adverse effects.[6] Nevertheless, the patient recovered and the blood cultures remained persistently negative after stopping colistin, thus emphasizing the potential role of starting with colistin plus double carbapenem and then simplifying to a less toxic regimen, such as ertapenem plus meropenem. Additional in vitro and in vivo studies are needed in order to provide more data on this innovative therapeutic approach.

In conclusion, this case highlights how an innovative regimen based on a short-course of colistin plus double carbapenems followed by double carbapenems might be considered as a valid and effective therapeutic strategy against severe infections caused by PDR *K. pneumoniae*.

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Conflict of interest: None to declare.

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