



Simultaneous colonization by *Escherichia coli* and *Klebsiella pneumoniae* harboring *mcr-1* in Brazil

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Abstract

Case presentation We present a case report of a woman, concurrently colonized by polymyxin-resistant *E. coli* and *K. pneumoniae*. A Brazilian female patient, in her mid-fifties, was hospitalized with schistosomiasis. During hospitalization, polymyxin-resistant *E. coli* and *K. pneumoniae* were isolated from surveillance cultures.

Methods Identification, antimicrobial susceptibility testings, PCR for *mcr-1*, plasmid transfer by conjugation and whole genome sequencing were performed.

Results *E. coli* ST744 and *K. pneumoniae* ST101 carrying *mcr-1* gene were described. Transconjugant *E. coli* was positive for *mcr-1* and IncX4 by PCR. The plasmid is a 33,304-base pair plasmid, and the *mcr-1* gene was the only antimicrobial resistance gene present in the plasmid.

Conclusions This study presents a case report of a hospitalized woman, concurrently colonized by *mcr-1*-harboring *E. coli* ST744, a different ST from previously described in Brazil, and a *K. pneumoniae* ST101.

Keywords Antimicrobial susceptibility · *mcr-1* · Resistance · Colistin

Polymyxins are peptide antimicrobials active against most Gram-negative bacteria, and multidrug resistance and

slowdown in antibiotic discoveries were responsible for the clinical return of this class of antimicrobials. Unfortunately, recent data have shown a significant increase in the number of isolates with high minimum inhibitory concentration for polymyxins against Gram-negative microorganisms, especially *Klebsiella pneumoniae* [1]. Until recently, polymyxin resistance had involved only vertical transmission due to chromosomal mutations, but a novel plasmidial colistin resistance gene (*mcr-1*) was identified, which can lead to horizontal transmission [2]. Most *mcr-1*-positive isolates origin from food animals and meat, with only a few clinical isolates reported [3]. The gene has previously been identified in Brazil [4], but this report is important due to co-colonization of *mcr-1*-harboring distinct species.

A female patient, in her mid-fifties, was hospitalized in March 2016 with schistosomiasis. She was born and lived in a rural town in the state of Paraíba, Northeastern Brazil. Her main diet was foods she produced, including chickens and pigs fed only locally sourced natural food. Three months before admission, she moved to São Paulo in search of medical care. In the third week after admission, she developed

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disorientation and a urine culture with ESBL-producing *E. coli*, susceptible to amikacin and carbapenems, but resistant to cephalosporins, ciprofloxacin, piperacillin/tazobactam and colistin. She had not used colistin previously. Surveillance swabs were collected 3 days after the diagnosis of the urinary infection. One colistin-resistant *E. coli* (TMS4) and two *K. pneumoniae* (TMS1 and TMS5) were isolated from surveillance cultures (Table 1). Infection control measures were implemented on an attempt to minimize the risk of dissemination: the patient was placed in a single room and healthcare workers were required to wear personal protective equipment, as recommended for contact precautions (gown and gloves during each contact with the patient or with the patient's environment). She was discharged after 5 days of meropenem. Two months after discharge, the patient was re-screened, and one surveillance culture using rectal swab in selective media was performed [5], which resulted negative for polymyxin-resistant microorganisms. We report the case of a patient simultaneously colonized by *E. coli* and *K. pneumoniae* harboring *mcr-1*. The report was approved for publication by the Research Ethics Committee of Hospital das Clínicas.

Identification was achieved using API 20E (bioMérieux, Marcy l'Etoile, France) and antibiotic susceptibility by Sensititre (Thermo Fisher Scientific, Cleveland, USA). PCR for *mcr-1* was performed as described previously [2]; amplification products were sequenced by Sanger method using MegaBACE 1000 (ABI 3730 DNA Analyser; Applied Biosystems, Alameda, CA). Plasmid transfer by conjugation was performed by broth mating experiments using *E. coli* C600 as the recipient strain. Transconjugant isolates were tested for *mcr-1* and incompatibility subgroup IncX4 by PCR [6]. Colistin-resistant isolates were whole genome sequenced (WGS) using two different platforms (MiSeq, Illumina, and Ion Torrent). De novo assemblies were carried out using SPAdes v.3.7.1. The plasmid harboring *mcr-1* was sequenced and analyzed with Geneious (v.9.1.8). Multilocus sequence type (ST) was determined from the assembled draft genome [7]. Unfortunately, it was not possible to evaluate the *E. coli* isolated from urine.

All isolates carried *mcr-1* confirmed by Sanger sequencing. *E. coli* TMS4 was able to conjugate and transconjugant *E. coli* was positive for *mcr-1* and IncX4 by PCR; *K. pneumoniae* isolates did not conjugate in vitro. Using Illumina and Ion Torrent assemblies, it was possible to determine the complete sequence of IncX4 plasmid from *E. coli* isolate (GenBank accession number MH298055). The plasmid, named pTMS4-1, was a 33,304-base pair plasmid and showed high nucleotide identity (99%) with pICBEC72H-mcr, a *mcr-1* bearing plasmid from an *E. coli* strain isolated in Brazil [4]. The *mcr-1* gene was the only antimicrobial resistance gene present in the plasmid.

Dissemination of *mcr-1* gene has been attributed to extensive use of polymyxins in livestock, since the presence of *mcr-1* positive strains is more frequent in animals or meat than in humans [4]. There are few data on colistin resistance in Brazil. In our hospital, colistin resistance is increasing among Gram-negative isolates, especially *K. pneumoniae* and *A. baumannii* [8]. This case report alerts to the horizontal transmission of polymyxin resistance mechanism involving two species: *E. coli* and *K. pneumoniae*. This patient was a Brazilian woman who always lived in the rural environment, had never travelled outside Brazil and worked in small-scale agriculture. She denied feeding her animals with industrialized food, and they were not available for testing for *mcr-1*.

Although there is to be no consensus about the sensitivity of the rectal swab culture (as it has been considered the gold standard to detect colonizing multiresistant microorganisms), this approach has been considered the main strategy to evaluate decolonization. In a previous study at our institution, a culture-based method was compared to a polymerase chain reaction method and the authors found high agreement between the two strategies [5].

E. coli ST744 has been isolated from humans, companion and wild animals, but rarely carry *mcr* genes [9]. *K. pneumoniae* ST101 has sometimes been described as multidrug resistant [10], but not yet carrying *mcr-1*. In general, *K. pneumoniae* is a rare carrier of *mcr-1*, and to our knowledge *mcr-1* co-colonization descriptions are scarce [11] and this is the first in Latin America. The finding of *mcr-1* gene in *K.*

Table 1 Antimicrobial susceptibility, antimicrobial resistance genotypes and colistin resistance-associated mutations identified by whole genome sequencing and PCR of *mcr-1*-positive Enterobacteriaceae isolates from a single patient with schistosomiasis complications

| Isolate | TMS4 | TMS1 | TMS5 |
|-------------------------------|---|--|--|
| Species | <i>E. coli</i> | <i>K. pneumoniae</i> | <i>K. pneumoniae</i> |
| Site | Rectal | Oropharyngeal | Rectal |
| Susceptibility ^a | | | |
| Colistin | > 4 (R) | > 4 (R) | > 4 (R) |
| Polymyxin B | > 4 (R) | > 4 (R) | > 4 (R) |
| Ceftazidime | > 16 (R) | > 16 (R) | > 16 (R) |
| Cefepime | ≤ 2 (S) | > 16 (R) | > 16 (R) |
| Ampicillin/Sulbactam | 32/16 (R) | > 64/32 (R) | > 64/32 (R) |
| Piperacillin/Tazobactam | ≤ 8/4 (S) | > 64/4 (R) | > 64/4 (R) |
| Aztreonam | 8 (S) | > 16 (R) | > 16 (R) |
| Imipenem | ≤ 1 (S) | ≤ 1 (S) | ≤ 1 (S) |
| Meropenem | ≤ 1 (S) | ≤ 1 (S) | ≤ 1 (S) |
| Doripenem | ≤ 0.5 (S) | ≤ 0.5 (S) | ≤ 0.5 (S) |
| Amikacin | 8 (S) | 8 (S) | 8 (S) |
| Gentamicin | > 8 (R) | > 8 (R) | ≤ 1 (S) |
| Tigecycline ^b | 0.5 (S) | 0.5 (S) | 0.5 (S) |
| Ciprofloxacin | > 2 (R) | > 2 (R) | > 2 (R) |
| Sulfamethoxazole/trimethoprim | > 4/76 (R) | > 4/76 (R) | > 4/76 (R) |
| Levofloxacin | > 8 (R) | > 8 (R) | > 8 (R) |
| MLST sequence type | 744 | 101 | 101 |
| Resistance genes | <i>aac(3)-VIa</i> | <i>aac(3)-IIId</i> | <i>aac(3)-IIId</i> |
| Aminoglycosides | <i>aac(6')Ib-cr</i> <i>aadA1</i> <i>aadA5</i> <i>aph(3')-Ia</i> <i>strA</i> , <i>strB</i> | <i>aac(6')Ib-cr</i> <i>aph(3')-Ia</i> <i>strA</i> , <i>strB</i> | <i>aac(6')Ib-cr</i> <i>aph(3')-Ia</i> <i>strA</i> , <i>strB</i> |
| Beta-lactams | <i>bla_{CMY-2}</i> <i>bla_{OXA-1}</i> <i>bla_{TEM-1B}</i> | <i>bla_{CTX-M-15}</i> <i>bla_{OXA-1}</i> <i>bla_{SHV-1}</i> <i>bla_{TEM-1B}</i> | <i>bla_{CTX-M-15}</i> <i>bla_{OXA-1}</i> <i>bla_{SHV-1}</i> <i>bla_{TEM-1B}</i> |
| Fluoroquinolones | <i>aac(6')Ib-cr</i> | <i>aac(6')Ib-cr</i> <i>oqxA</i> , <i>oqxB</i> | <i>aac(6')Ib-cr</i> <i>oqxA</i> , <i>oqxB</i> |
| Fosfomycin | – | <i>fosA</i> | <i>fosA</i> |
| Sulphonamides | <i>sul1</i> , <i>sul2</i> | <i>sul2</i> | <i>sul2</i> |
| Tetracyclines | <i>tet(B)</i> | <i>tet(D)</i> | <i>tet(D)</i> |
| Trimethoprim | <i>dfrA14</i> , <i>dfrA17</i> | <i>dfrA14</i> | <i>dfrA14</i> |
| Colistin | <i>mcr-1</i> | <i>mcr-1</i> | <i>mcr-1</i> |
| <i>pmrA</i> | S31T; N128I; S144G | T245A | T157P; T245A |
| <i>pmrB</i> | R2H; D123E; G283D; I351V | A217V | A217V |
| <i>pmrC</i> (<i>eptA</i>) | F14L; S15A; T21A; V26I; C27Y; V39A; G69S; T106A; R123Q; L130F; I137L; T147A; I163V; S211L; I217V; G232E; Q257P; V332A; I341V; G348D; S373G; L376V | G25S; I138V; Q319R | G25S; I138V; Q319R |
| <i>eptB</i> | V505A; R558K | – | – |
| <i>phoP</i> | L44I | – | – |
| <i>phoQ</i> | H6R | – | – |
| <i>mgrB</i> | – | M1V | M1V |
| <i>lpxM</i> | C45R; V66A; I94M | S253G | S253G |
| <i>yciM</i> | Y72H | – | – |
| <i>acrB</i> | N596H | – | – |

According to Clinical and Laboratory Standards Institute breakpoints

S susceptible, I intermediate, R resistant

^aMinimal inhibitory concentrations (mg/L)

^bFor Tigecycline, Food and Drug Administration breakpoints were used

pneumoniae is particularly important because it is frequently multidrug resistant [12]. Our study presents a case report of a woman concurrently colonized by *mcr-1*-harboring *E. coli* ST744, a different ST from previously described in Brazil, and a *K. pneumoniae* ST101 for the first time.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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