Received Date : 07-Mar-2016
Revised Date : 26-Apr-2016
Accepted Date : 16-May-2016
Article type : Case Report

TID-16-C-041.R1, Received 7 March 2016, revised 26 April 2016, accepted for publication 16 May 2016

Case report

Successful treatment of a disseminated infection with extensively drug-resistant *Klebsiella pneumoniae* in a liver transplant recipient with a fosfomycin-based multidrug regimen

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Running Title: Mills et al: Fosfomycin for donor-derived CRKP infection

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Abstract: Donor-derived infections with multidrug-resistant gram-negative bacteria are associated with poor outcomes, in part due to limited treatment options. Here we describe a case of donor-derived, disseminated infection with colistin-resistant, carbapenemase-producing *Klebsiella pneumoniae* in a liver transplant recipient that was cured with addition of intravenous fosfomycin to a multidrug regimen, in conjunction with aggressive surgical source control. Intravenous fosfomycin represents a promising adjunctive agent for use in treatment of extensively drug-resistant infections in immunocompromised hosts.

Key words: carbapenem-resistant *Klebsiella pneumoniae* (CRKP); orthotopic liver transplantation (OLT); fosfomycin; extremely drug-resistant (XDR)

Extensively drug-resistant (XDR) gram-negative bacilli possess resistance to all but 1 or 2 antibiotic classes (1). Infections caused by XDR Enterobacteriaceae are associated with high mortality rates, particularly in immunocompromised hosts (2, 3). Treatment options are limited for these infections and the optimal treatment for these infections is unknown, especially in those at greatest risk for poor outcomes. Here we describe the successful treatment of a donor-derived, disseminated XDR *Klebsiella pneumoniae* infection in a liver transplant recipient after addition of intravenous (IV) fosfomycin to a multidrug regimen.

Case report

A 68-year-old woman, with a history of decompensated cirrhosis and recurrent ascites due to chronic hepatitis C and prior alcohol dependence, was admitted from home for orthotopic liver transplantation. Before transplantation, the patient was stable without recent fevers or other symptoms of acute illness.

The donor was a previous lung transplant recipient who died after a prolonged hospital stay because of respiratory failure requiring mechanical ventilation, and who had a prior history of respiratory colonization with carbapenem-resistant *K. pneumoniae* (CRKP); a recent bloodstream infection was not present.

Peri-operatively, the patient received ampicillin/sulbactam 1.5 g every 6 h. Intraoperative complications did not occur; the patient was extubated immediately postoperatively and transferred to the floor on hospital day 3. Oral trimethoprim-sulfamethoxazole and acyclovir were initiated postoperatively for prophylaxis of infection. Immunosuppressive medications included a 200 mg methylprednisolone taper, azathioprine 100 mg daily, and tacrolimus 2 mg twice daily.

On hospital day 3, the patient was started on empirical IV gentamicin following report of positive *K. pneumoniae* growth from donor lung tissue cultures obtained at the time of explantation. Subsequent susceptibility testing revealed resistance to all tested antimicrobial agents, with the exception of colistin and tigecycline, and intermediate susceptibility to gentamicin. On hospital day 5, she was transferred to the surgical intensive care unit for tachycardia, hypotension, leukocytosis and renal dysfunction. Blood cultures obtained on hospital day 5 grew CRKP from 3 of 4 vials with an identical

susceptibility pattern to the donor CRKP isolate (Table 1). On hospital day 6, the patient was intubated for respiratory failure. Gentamicin was discontinued and the following regimen was initiated: meropenem 2 g every 8 h by continuous infusion, colistin with dose adjusted for impaired renal function, and tigecycline 100 mg loading dose followed by 50 mg every 12 h.

Repeat blood cultures on hospital day 6 grew CRKP from 3 of 6 vials. Chest x-ray showed new perihilar, left upper, and left lower lobe opacities consistent with multifocal pneumonia and sputum culture grew CRKP.

The patient was extubated on hospital day 8 and surveillance blood cultures from hospital days 8, 10, and 11 remained negative, but the patient's leukocytosis continued to worsen. Computed tomography scan of the abdomen and pelvis with oral contrast did not show any fluid collections or evidence of bowel leak.

The patient was started on continuous renal replacement therapy for anuric renal failure and inability to tolerate hemodialysis because of hypotension. Serum nucleic acid testing for cytomegalovirus was positive and the patient was started on IV ganciclovir. Because of worsening liver function tests and recurrent ascites, the patient was re-listed for orthotopic liver transplant. On hospital day 28, the patient underwent simultaneous liver and kidney transplant. All central venous catheters were removed prior to surgery and placed at new sites in the operating room (OR).

The patient did not experience any intraoperative complications and had vasopressors weaned within 24 h of surgery. On hospital day 29, blood cultures were drawn because of a new fever, and 2 of 4 vials were positive for CRKP. The patient was continued on meropenem and colistin, and tigecycline was again added to the regimen on hospital day 37.

The patient underwent exploratory laparotomy and washout of infected ascites on hospital day 42. Ascites cultures grew CRKP as well as vancomycin-resistant *Enterococcus faecium*. Daptomycin and gentamicin were subsequently added to the antibiotic regimen. The patient returned to the OR on hospital day 50 for repeat exploratory laparotomy, lysis of adhesions, and evacuation of intra-abdominal abscesses and hematoma as well as abdominal irrigation. Ascitic fluid cultures again grew CRKP, with colistin MIC > 256 by Etest (Table 2). Blood cultures remained persistently positive

between hospital days 29 and 50. After confirming susceptibility by disk diffusion and obtaining investigational new drug approval, IV fosfomycin 8 mg every 12 h was added to the existing drug regimen on hospital day 52. The patient underwent peritoneal lavage of infected ascites on hospital day 53 (no cultures sent), and abdominal wall debridement, lysis of adhesions, and intra-abdominal lavage on hospital day 59, with OR cultures again positive for CRKP. Six sets of blood cultures from hospital days 53–56 remained negative for bacterial growth. Repeat disk diffusion testing on the CRKP isolate from hospital day 59 revealed resistance to fosfomycin.

Over the subsequent 2–3 weeks, the patient clinically improved, with resolution of leukocytosis and improvement in renal function, with discontinuation of renal replacement therapy. Repeat computed tomography of the abdomen and pelvis on hospital day 86 showed small pelvic abscess that was not amenable to drainage. Ascitic cultures from therapeutic paracentesis on hospital day 87 were negative for bacterial growth. IV fosfomycin was discontinued on hospital day 109. Meropenem, gentamicin, and tigecycline were discontinued on hospital day 137.

The patient was successfully weaned from mechanical ventilation and discharge to a long-term acute care hospital on hospital day 206. She remains alive and well without any recurrence of CRKP infection 15 months since her second transplant.

Microbiology

Bacterial identification was performed via VITEK MS MALDI-TOF (bioMerieux, Marcy L'Etoile, France), with routine susceptibility testing performed by Vitek2 (bioMerieux, Durham, North Carolina USA) and interpreted according to Clinical Laboratory Standards Institute (CLSI) guidelines. Susceptibility testing for colistin and tigecycline were performed by Etest (bioMerieux, Durham). Fosfomycin susceptibility testing was performed by the disk diffusion method on CRKP isolates from hospital days 5 and 59. Zone diameter cutoffs were determined by CLSI interpretive criteria for urinary isolates of *Escherichia coli*. Polymerase chain reaction for carbapenemases was performed on the initial positive blood culture from hospital day 5. It confirmed presence of $bla_{\text{KPC-3}}$ allele and *wzi* sequencing identified *wzi*-168. Metallo-beta-lactamases were not found.

Discussion

Our case demonstrates several unique aspects, including donor derivation despite the absence of blood stream or disseminated infection or involvement of the transplanted organ, and the impressive improvement following initiation of IV fosfomycin.

Recent reports highlighted the vulnerability of solid organ transplant (SOT) recipients to infections due to multidrug-resistant (MDR) gram-negative bacteria. The convergence of multiple risk factors, including prolonged hospitalization, selective pressure of antibiotic exposure, immunosuppression, and high rates of device utilization conspire to render transplant recipients highly susceptible to acquisition of MDR organisms (4). In our case, the donor characteristics, including prior known respiratory colonization with CRKP, were balanced with the patient's advanced disease state. In the absence of positive blood cultures or known hepatic infection in the donor, the decision was made to proceed with liver transplantation.

Post-transplant infections with carbapenem-resistant Enterobacteriaceae (CRE) are associated with poor outcomes. A study of CRE infections after liver transplantation described a one-year survival rate of 23% versus 86% for those with CRE infections versus those without, resulting in a hazard ratio of 4.9 (5). A recent nationwide Italian study of gram-negative bacterial infection after solid organ transplantation found that recipients with ≥ 1 positive culture for carbapenem-resistant gram-negative bacteria had a 10.23-fold increased mortality rate versus those who did not have carbapenem-resistant gram-negative bacteria isolated (6).

Optimal therapy for systemic CRE infections in SOT recipients is not well-defined. Current guidelines by the American Society of Transplantation recommend optimizing source control and use of ≥ 2 of the following agents: colistin, tigecycline, aminoglycosides (if susceptible), and high-dose, prolonged infusion of carbapenems (level II-3 data) (4). The guidelines do not specifically address treatment of colistin-resistant CRE infections and very few data exist on management of these infections in SOT recipients.

Fosfomycin binds to the enzyme UDP-N-acetylglucoasmine enolpyruvyl transferase (MurA), inhibiting the formation of N-acetylmuramic acid, resulting in disruption of peptidoglycan assembly (7). It is a bactericidal agent with a broad-spectrum

of activity against gram-positive and gram-negative aerobic bacteria, including methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecium*, and CRE. Fosfomycin is a small, hydrophilic molecule that achieves high levels of tissue penetration, including central nervous system, and has minimal drug-drug interactions (8). Fosfomycin is generally well tolerated, with hypokalemia being the most common adverse effect. The IV formulation contains 14.4 meq of sodium for every 1 g of fosfomycin, which may potentiate volume overload in at-risk patients, such as those with congestive heart failure or renal insufficiency (9). Standard IV dosing for fosfomycin is 12–24 g daily, administered in divided doses every 6–8 h. Dose reductions are necessary for creatinine clearance < 50 mL/min. Currently unavailable in the United States, IV fosfomycin is available on a compassionate use basis from the European branch of Sanofi, under the aegis of the emergency Investigational New Drug application process.

Fosfomycin resistance mainly occurs through chromosomal mutations resulting in decreased drug uptake due to mutations in genes affecting the active transport systems (9). European Committee on Antimicrobial Susceptibility Testing breakpoints for IV fosfomycin for treatment of Enterobacteriaceae are as follows: susceptible≤ 32 m g/L; resistant > 32 mg/L. CLSI breakpoints exist only for the oral formulation of fosfomycin for treatment of *E. coli* urinary tract infections, with susceptibility defined as < 256 mg/L by agar dilution or an inhibition zone≥ 16 mm by disk diffusion . As fosfomycin works through a unique mechanism of action, cross-resistance to other classes of antibiotics has not been documented, and prior data show evidence of a synergistic effect when used in combination with beta-lactams or aminoglycosides (10). Notably significant drug interactions with standard immunosuppressive agents are not present.

A recent investigation of CRE isolates in Germany found 72% of strains to be susceptible to fosfomycin by agar dilution testing (11). Studies on treatment of CRE with IV fosfomycin are small but show promising results. A retrospective study of patients receiving IV fosfomycin in combination with colistin or tigecycline for XDR, fosfomycin-susceptible, *K. pneumoniae* and *Pseudomonas aeruginosa* infections in Greek intensive care units noted successful outcomes in 54% of patients at day 14, with failure, indeterminate outcome, and superinfection occurring in 33%, 6%, and 6% of cases. Emergence of fosfomycin resistance occurred in 3 cases (12).

We postulate that our patient's clinical improvement was a result of a combination of aggressive source control, reduction of immunosuppression, and addition of IV fosfomycin to the patient's pre-existing antimicrobial regimen. Although we cannot know with certainty that fosfomycin played a critical role in the patient's improvement, we observed microbiologic response (clearance of blood cultures) within 48 h of addition of fosfomycin, after 3 weeks of sustained bacteremia on 3-drug therapy, followed by sustained clinical improvement. The significance of fosfomycin resistance identified on repeat disk diffusion testing after 1 week of therapy is unclear, as the interpretative criteria for susceptibility testing did not accurately reflect the pathogen or site of infection in this case, limiting the ability to extrapolate *in vivo* activity. However, use of fosfomycin in combination with another active antimicrobial is recommended to limit the risk of emergence of resistance.

Conclusion

MDR gram-negative bacterial infections in SOT recipients are associated with high mortality rates. Treatment of infections caused by XDR Enterobacteriaceae remains challenging because of limitations of currently available therapeutic options. IV fosfomycin is an agent with a unique mechanism of action, minimal drug-drug interactions, good tissue penetration, and an excellent safety profile, which may be valuable as part of a multidrug regimen to treat disseminated XDR Enterobacteriaceae infections in immunocompromised hosts.

Acknowledgments:

Support: Funds and facilities provided by the Cleveland Department of Veterans Affairs, the Veterans Affairs Merit Review Program award number 1I01BX001974, and the Geriatric Research Education and Clinical Center VISN to RAB supported the laboratory experiments in this work.

This work was also supported by funds from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award numbers R01AI063517 and R01AI100560 to RAB, and through the Antibiotic Resistance

Leadership Group under National Institutes of Health award number UM1AI104681, and NIH award number R01AI072219.
Funding organizations were not involved in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.
Disclaimer: The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the Department of Veterans Affairs

Potential conflicts of interest: None to report.

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Figure Legend:

Fig. 1. Timeline of (A) antibiotic administration, and (b) surgical procedures and blood and peritoneal fluid culture results. OLT, orthotopic iver transplantation; DDKT, deceased-donor kidney transplant; LOA, lysis of adhesions; I&D, incision and drainage; *K., Klebsiella.*

Minimum inhibitory concentrations (MICs) and susceptibility results of select antibiotics to initial CRKP isolate

Agent	MIC	Interpretation	Testing method
Ampicillin	≥32	R	Vitek 2
Piperacillin-tazobactam	≥128	R	Vitek 2
Ceftazidime	≥64	R	Vitek 2
Cefepime	4	R	Vitek 2
Aztreonam	≥32	R	Vitek 2
Meropenem	≥16	R	Vitek 2
Amikacin	≥64	R	Vitek 2
Gentamicin	1	I	Vitek 2
Tobramycin	≥16	R	Vitek 2
Levofloxacin	≥8	R	Vitek 2
TMX-SMP	8	R	Vitek 2
Tigecycline	1.5	S	E-test
Colistin	0.25	--	E-test

CRKP, carbapenem-resistant Klebsiella pneumoniae; R, resistant; I, intermediate; TMX-SMP, trimethoprim-sulfamethoxazole; S, sensitive .

Table 1

Minimum inhibitory concentrations of select agents to CRKP isolates over time

Agent	Hospital day 5	Hospital day 32	Hospital day 42	Hospital day 50
Tigecycline	1.5	1.5	---	4
Colistin	0.25	16	64	>256

CRKP, carbapenem-resistant Klebsiella pneumoniae.

Table 2

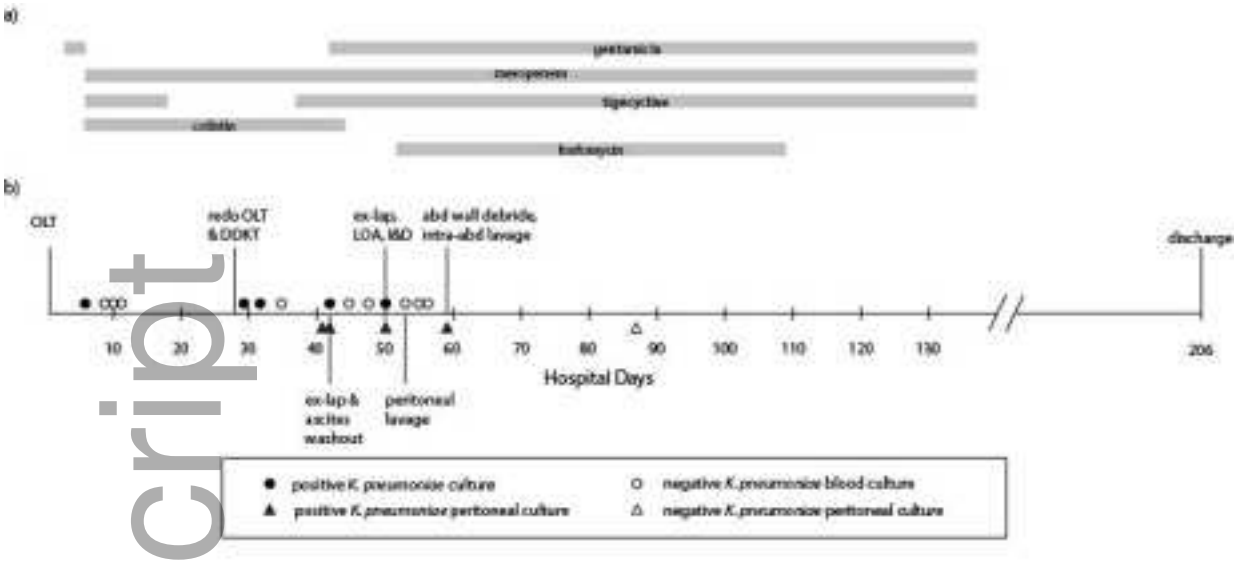

Figure 1: Timeline of antibiotic administration (a), surgical procedures and blood and peritoneal fluid culture results (b). OLT = orthotopic liver transplantation, DDKT = deceased donor kidney transplant, LOA = lysis of adhesions, I&D = incision and drainage.

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