Case Report

First Report of a Verona Integron-Encoded Metallo-β-Lactamase-Producing *Klebsiella pneumoniae* Infection in a Child in the United States

Pranita D. Tamma,[1] Nuntra Suwantarat,[2,3] Susan D. Rudin,[4] Latania K. Logan,[6,7] Patricia J. Simner,[2] Laura J. Rojas,[4,8,9] Maria F. Mojica,[4,8,10] Karen C. Carroll,[2] and Robert A. Bonomo[5,8–11]

[1]Divisions of Pediatric Infectious Diseases, and [2]Medical Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland; [3]Chulabhorn International College of Medicine, Thammasat University, Pathumthani, Thailand; [4]Research Service, and [5]Geriatrics Research, Education and Clinical Center, Louis Stokes Cleveland Department of Veterans Affairs Medical Center, Ohio; [6]Section of Pediatric Infectious Diseases, Department of Pediatrics, Rush University Medical Center, Rush Medical College, Chicago, Illinois; [7]Louis Stokes Cleveland Department of Veterans Affairs Medical Center, Departments of [8]Medicine, [9]Molecular Biology and Microbiology, [10]Biochemistry, and [11]Pharmacology, Case Western Reserve University School of Medicine, Cleveland, Ohio

Corresponding Author: Pranita D. Tamma, MD, MHS, Johns Hopkins University School of Medicine, 200 N Wolfe St, Ste 3149, Baltimore, MD 21287. E-mail: ptamma1@jhmi.edu.

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We report the first case of a child in the United States infected with an organism producing a Verona Integron-Encoded Metallo-β-Lactamase. This child succumbed to a ventilator-associated pneumonia caused by a *Klebsiella pneumoniae* producing this resistance mechanism.

Key words. carbapenemase; CRE; metallo-β-lactamase; pediatrics; VIM.

CASE REPORT

A full-term, 2-year-old male with congenital hydrocephalus requiring chronic invasive mechanical ventilation was transferred from a tertiary care hospital in Kuwait to a pediatric intensive care unit (PICU) in Maryland for ventriculoperitoneal shunt placement in May 2014. Upon arrival in the United States, he did not have evidence of an active infection. A rectal surveillance swab was obtained at the time of PICU admission because he received medical care abroad, per recommendations from the United States Centers for Disease Control and Prevention [1].

The rectal swab was processed in real time and inoculated into tryptic soy broth containing a 30-μg ceftriaxone disk and incubated at 37°C. After overnight incubation, 100 μL of broth sample with visible turbidity was plated on MacConkey agar with a 30-μg ceftriaxone disk and incubated at 37°C overnight. All isolates growing within 19 mm of the ceftriaxone disk (resistant zone diameter) underwent routine identification and antimicrobial susceptibility testing using the Phoenix Automated System (BD Diagnostics, Sparks, Maryland). An isolate of *Klebsiella pneumoniae* was recovered. The antibiotic minimum inhibitory concentrations are shown in Table 1. Because the isolate was carbapenem resistant, further phenotypic and molecular testing was performed. The organism was both modified Hodge test and metallo-β-lactamase (MβL) Etest positive (bioMérieux, Durham, North Carolina).

Upon identification of a carbapenemase-producing *K pneumoniae* from the admission rectal surveillance swab, the child was placed on contact precautions for the duration of his hospitalization. He remained hospitalized for 5 months, and weekly rectal surveillance swabs remained positive for this organism throughout the hospitalization. Active rectal surveillance swabs at the time of admission and weekly thereafter for all children hospitalized in the PICU were conducted [2]. No transmission of this resistant isolate to other children occurred. The patient developed a ventilator-associated pneumonia with a bronchoalveolar lavage fluid sample identifying the same carbapenem-resistant *K pneumoniae* isolate during his fifth month of hospitalization. Because he was known to be colonized with a carbapenemase-producing *Enterobacteriaceae*, he was empirically treated with extended-infusion meropenem and amikacin. Regrettably, on his fourth day of

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Table 1. Results of Phenotypic and Antibiotic Susceptibility Testing of *Klebsiella pneumoniae* Isolates Recovered From Rectal Swabs and Bronchoalveolar Lavage Fluid From a Child and an *Escherichia coli* Isolate After Conjugation

Source of Isolate	Modified Hodge Test	Metallo-β-lactamase Etest	Minimum Inhibitory Concentration (μg/mL)[a]														
			Aztreonam	Ceftriaxone	Cefepime	Piperacillin	Ertapenem	Imipenem	Meropenem	Doripenem	Ciprofloxacin	Gentamicin	Amikacin	Tobramycin	Tetracycline	Tigecycline[b]	Colistin[c]	Fosfomycin[d]
Admission rectal swab	Positive	Positive	8 (I)	>32 (R)	16 (R)	>64 (R)	>8 (R)	>8 (R)	>8 (R)	>2 (R)	>2 (R)	>8 (R)	<8 (S)	>8 (R)	>8 (R)	2 (I)	0.12 (S)	16 (S)
Rectal swab 4 months into hospitalization[e]	Positive	Positive	8 (I)	>32 (R)	16 (R)	>64 (R)	>8 (R)	>8 (R)	>8 (R)	>2 (R)	>2 (R)	>8 (R)	<8 (S)	>8 (R)	>8 (R)	4 (R)	0.12 (S)	16 (S)
Bronchoalveolar lavage fluid sample obtained 5 months into hospitalization	Positive	Positive	8 (I)	>32 (R)	16 (R)	>64 (R)	>8 (R)	>8 (R)	>8 (R)	>2 (R)	>2 (R)	>8 (R)	<8 (S)	>8 (R)	>8 (R)	4 (R)	0.12 (S)	16 (S)
E coli strain	Negative	Negative	≤1 (S)	≤1 (S)	≤1 (S)	≤1 (S)	≤0.5 (S)	≤1 (S)	≤1 (S)	≤1 (S)	≤0.5 (S)	≤1 (S)	≤2 (S)	≤1 (S)	–	≤0.12 (S)	–	–
E coli J53 transconjugate strain	Positive	Positive	8 (I)	>32 (R)	2 (S)	>64 (R)	>8 (R)	8 (R)	2 (I)	2 (I)	<0.5 (S)	8 (I)	<8 (S)	>8 (R)	>8 (R)	<0.25 (S)	0.06 (S)	2 (S)

[a]Susceptibility results are based on Clinical and Laboratory Standards Institute (CLSI) recommended breakpoints when available.
[b]No tigecycline CLSI interpretive criteria available; European Committee on Antimicrobial Susceptibility Testing (EUCAST) defines tigecycline susceptibility as ≤1 μg/mL.
[c]No colistin CLSI interpretive criteria available for *Enterobacteriaceae*, EUCAST defines colistin susceptibility as ≤2 μg/mL.
[d]No fosfomycin CLSI interpretive criteria available for *Enterobacteriaceae* outside of urinary tract isolates, EUCAST defines fosfomycin susceptibility as ≤32 μg/mL.
[e]Additional weekly rectal swabs are not included to simplify table but showed identical phenotypic testing and susceptibility patterns.

antibiotic therapy for ventilator-associated pneumonia, the patient died.

The genetic relatedness of our patient's rectal surveillance isolates and his clinical isolate was assessed by repetitive sequence-based polymerase chain reaction (rep-PCR) and multilocus sequence typing (MLST). Isolates with ≥95% similarity were considered to be of the same rep-PCR type. Sequences of 7 housekeeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, and *tonB*) were identified using the Pasteur MLST database (http://www.pasteur.fr/recherche/genopole/PF8/mlst). Rectal surveillance swabs and the clinical isolate were identical by rep-PCR and identified as sequence type 14 (ST14) by MLST.

To identify the β-lactamase genes associated with the carbapenem-resistant phenotype, the Check-MDR CT103 XL assay (CheckPoints, Wageningen, Netherlands) was performed on the surveillance and clinical isolates. We selected this platform because the Check-MDR CT103 XL assay combines PCR amplification and deoxyribonucleic acid microarray technologies for the detection of extended-spectrum β-lactamase (ESBL) genes, plasmid-mediated AmpC β-lactamase genes (pAmpC), and an extended panel of carbapenemase genes (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, $bla_{OXA-48-like}$, bla_{GES}, bla_{GIM}, bla_{SPM}, $bla_{OXA-23-like}$, $bla_{OXA-24/40-like}$, $bla_{OXA-58-like}$). All tested isolates contained both the carbapenemase bla_{VIM} and the pAmpC $bla_{CMY-2-group}$. They were confirmed to be bla_{VIM-4} and bla_{CMY-4} by sequencing [3].

Conjugation experiments, using a variation of filter mating, succeeded in transferring the bla_{VIM-4}-containing plasmid to the susceptible recipient strain, *Escherichia coli* J53 (Figure 1). The transconjugant (TC-18) was verified to be *E coli* and not breakthrough *K pneumoniae*, and antimicrobial susceptibility testing was performed (Table 1). S1 nuclease pulsed-field gel electrophoresis indicated that the parent *K pneumoniae* strain contained 3 plasmids and the transconjugant strain contained only 1 plasmid (Figure 1). Subsequent southern hybridization with the bla_{VIM} probe demonstrated that the 188-kb plasmid common to both strains contained the bla_{VIM} gene. Further PCR analysis indicated that the bla_{CMY-4} gene was transferred along with the bla_{VIM-4} gene, thereby suggesting it was also contained in the transferable 188-kb plasmid. Polymerase chain reaction-based plasmid replicon typing identified 2 of the 3 replicon types present in the *K pneumoniae* strain (A/C and FIIA; the third type undetermined) and that the transferable 188-kb plasmid was the A/C replicon.

DISCUSSION

We report the first case of a Verona integron-encoded MβL (VIM) infection in a pediatric patient in the United States.

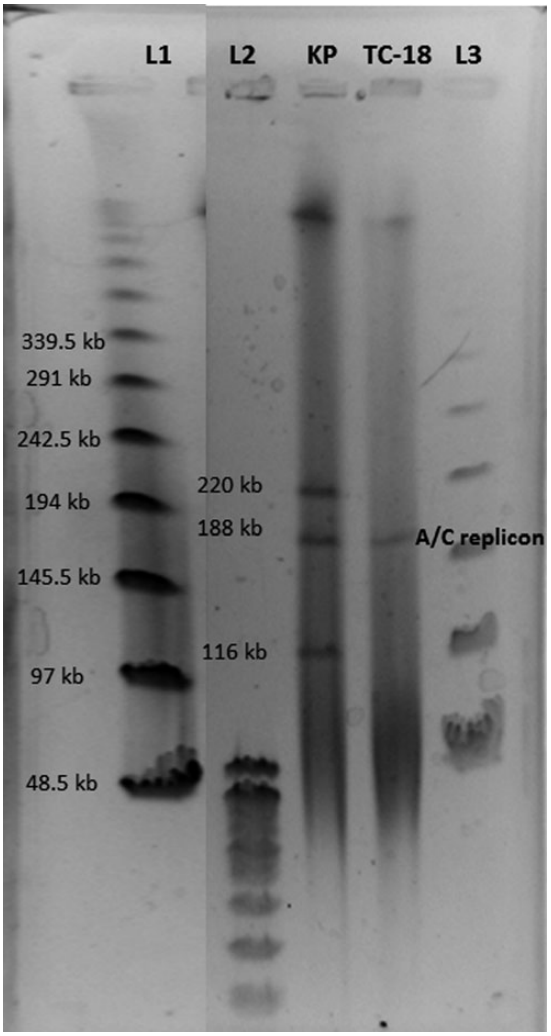

Figure 1. Southern blotting results for the metallo-β-lactamase-producing *Klebsiella pneumoniae* (KP) and the *Escherichia coli* transconjugant (TC-18). The bla_{VIM-4} and bla_{CMY-4} genes were transferable to the *E coli* isolate via the A/C replicon (188 kb). L1 and L3 represent the CHEF deoxyribonucleic acid (DNA) size standards, and L2 is the DNA standard size 8–48 kb (Bio-Rad, Hercules, California).

Our patient remained colonized with this organism for at least 5 months and subsequently succumbed to a ventilator-associated pneumonia from this MβL-producing *K pneumoniae*. Other notable members of the MβL class include the "active on imipenems" (IMP) and New Delhi metallo-β-lactamases (NDM). Members of the different MβL subclasses differ not only in their high degree of sequence diversity, but also in the structure of their active sites [4]. The MβLs hydrolyze penicillins, cephalosporins, and carbapenems using zinc. In contrast to the class A and D serine-β-lactamases, notably the *K pneumoniae* carbapenemase and oxacillinase-types, the monobactam antibiotic aztreonam is spared from hydrolysis by the MβLs [4]. Like other carbapenemase-producers, MβLs can cause infections resulting in considerable morbidity and mortality [4].

Although MβL were first identified in the 1960s [4], they only came to prominence in the 1990s. Verona integron-encoded MβL-1 was discovered in *Pseudomonas aeruginosa* in 1996 [5]. Currently, more than 25 VIM allotypes belonging to 3 sublineages are recognized. bla_{VIM} genes are predominantly found in *P aeruginosa* and other Gram-negative nonfermenters, but they are increasingly being reported in a number of *Enterobacteriaceae* [4]. Gram-negative bacteria possessing bla_{VIM} are still mostly confined to the Mediterranean basin and Middle East. In one study in Greece, among 178 consecutive *K pneumoniae* bloodstream isolates, approximately 40% were bla_{VIM-1} positive [6]. The rapid dissemination of bla_{VIM} in Greece has led to an endemic situation within a short period of time [6]. Imported cases involving acquired VIM carbapenemases are now described in both children and adults throughout Europe, Asia, and South America [4]. There have been a few isolated cases of infections caused by organisms producing VIMs reported in adults in the United States [7–10]. The first US case of an Enterobacteriaceae that produced a bla_{VIM} was identified in 2010 [9]. This case involved a woman who required 12 days of hospitalization in Greece after becoming ill on a cruise, and upon return to the United States she developed septicemia from a VIM-producing *K pneumoniae*. In 2015, there was a report of 6 neonates in a neonatal intensive care unit in Kentucky becoming colonized with *Enterobacteriaceae* containing the bla_{VIM} gene. Fortunately, no clinical infections were reported, but this cluster highlights the potential for rapid propagation of this resistance mechanism between critically ill patients [11].

In general, the dissemination of acquired MβL genes among Gram-negative organisms is mediated by mobile gene cassettes inserted into integrons [4]. The bla_{VIM} gene is generally found to be part of a type 1 integron. These integrons are usually harbored by transferable plasmids with a high capacity for horizontal transmission. Most integrons containing gene cassettes for MβL also harbor additional gene cassettes carrying resistance determinants for a wide variety of antibiotic classes. Therefore, MβL-producing organisms frequently exhibit complex multidrug-resistance. Although aztreonam is not hydrolyzed by MβLs, its activity is often impaired by additional acquired mechanisms of resistance by organisms producing MβLs, such as ESBL or AmpC β-lactamase enzymes. This was likely the case with our patient whose *K pneumoniae* isolate was nonsusceptible to aztreonam and carried a pAmpC gene (bla_{CMY-4}). Of concern, unlike the serine carbapenemases, there are very few novel agents under development with the potential to inhibit the production of MβLs [12, 13].

Metallo-β-lactamase-producing bacteria have caused a number of outbreaks throughout the world. An outbreak in an Australian ICU involved the MβL gene bla_{IMP-4}, thought to be imported from an East Asian patient [13]. After the first detection of bla_{IMP-4} in a *P aeruginosa* isolate, the bla_{IMP-4} gene was subsequently identified in hospital-acquired isolates of Gram-negative pathogens of 5 different species, including several *Enterobacteriaceae*, over a 7-month period [14]. This outbreak was particularly concerning as it suggested that intraspecies and interspecies gene transfer plays a major role in the dissemination of MβL within healthcare settings. This case highlights the importance of screening patients for carbapenemases who have had previous healthcare exposure in endemic regions of the world. Because our patient was screened upon admission to the ICU, we believe the early recognition of colonization with an MβL-producing organism and the swift application of contact precautions likely prevented dissemination of this organism in our PICU.

CONCLUSIONS

In summary, our case reminds us that MβL-producing organisms with complex multidrug-resistant phenotypes can be a formidable clinical challenge. It is likely only a matter of time until they become endemic in pediatric healthcare settings. Identifying imported MβLs is important to prevent this resistance mechanism from becoming endemic in the United States, as it is in several other regions of the world.

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