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Molecular diversity in mechanisms of carbapenem resistance in paediatric Enterobacteriaceae

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ABSTRACT

Development of carbapenem resistance in Enterobacteriaceae has impacted Clinical and Laboratory Standards Institute (CLSI) guidelines, infection control approaches and treatment strategies. The clinical, phenotypic and genotypic characteristics of carbapenem-resistant Enterobacteriaceae (CRE) infections at paediatric referral centres are not well described. CRE were identified through the clinical microbiology laboratory at Seattle Children's Hospital (Seattle, WA). Clinical data were retrieved from medical records. Resistance testing, polymerase chain reaction (PCR) for resistance determinants, and *Escherichia coli* transformation were carried out for each isolate. Multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) were used to characterise strain relatedness. PCR amplification and sequencing as well as sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) were used to investigate porin alterations. Six CRE isolates were identified between 2002 and 2010. Significant molecular diversity was documented in their mechanisms of resistance, including plasmid-mediated serine carbapenemase (KPC) and metallo-β-lactamase (IMP), chromosomally encoded β-lactamase (SME) and porin alterations with extended-spectrum β-lactamases. Patients had underlying health conditions and were from geographically diverse regions. In one case, PFGE of serial isolates documented the development of resistance in a previously susceptible strain. Molecular investigation of this strain identified insertion of the genetic mobile element insertion sequence IS*Ecp1* in the *ompK36* gene, conferring a functional porin alteration as demonstrated by SDS-PAGE. This is the first description of porin disruption by IS*Ecp1* in a CTX-M-15-positive isolate. This is the largest report of paediatric CRE to date. This diverse description of demographic, phenotypic and molecular characteristics highlights the challenge of CRE infections in high-risk paediatric patients and that attention to emerging resistance mechanisms (including membrane alteration) at paediatric referral centres is essential.

1. Introduction

The rapid emergence and spread of carbapenem resistance amongst Gram-negative pathogens has challenged clinicians to devise effective empirical and definitive antibiotic strategies for individual patients as well as institutional protocols [1,2]. Enterobacteriaceae are responsible for a large proportion both of hospital- and community-acquired infections and affect a wide variety of hosts. The effect of increasing rates of carbapenem resistance amongst Enterobacteriaceae necessitates a comprehensive study of the clinical features and molecular epidemiology of these challenging infections [3,4]. These characteristics are poorly described for paediatric referral centres, which are institutions contending with high-risk patients and increasing numbers of antibiotic-resistant pathogens.

Carbapenem resistance can result from the acquisition of plasmid or chromosomal resistance genes encoding serine carbapenemase or metallo-β-lactamase enzymes and efflux pumps, or by alteration of porin expression in combination with an AmpC- or extended-spectrum β-lactamase (ESBL)-type enzyme [5]. The nature of the responsible resistance determinant(s) may affect the dynamics of spread, the ability to detect the associated resistance phenotype in the clinical laboratory and the organism's susceptibility to treatment with alternative antibiotics [6]. Defining the clinical and molecular characteristics of carbapenem-resistant isolates will elucidate the breadth of emerging resistance and provide data to help guide current efforts to detect these strains and control their spread. Focused study is important as new Clinical and Laboratory Standards Institute (CLSI) guidelines for identifying carbapenem resistance are released (CLSI M100-S21) and institutions

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implement changes that will affect infection control, surveillance and patient care.

There are few studies of paediatric infections due to carbapenem-resistant Enterobacteriaceae (CRE). Here we describe the clinical and molecular details of the largest paediatric case series to date, comprising six cases of carbapenem resistance in patients seen between 2002 and 2010 at Seattle Children's Hospital (SCH), a 250-bed tertiary care centre in Seattle, WA.

2. Case summaries

2.1. Case 1

A 16-year-old Native American male from Washington was admitted for treatment of acute myelogenous leukaemia (AML). On Day 13 of hospitalisation, whilst receiving chemotherapy, he developed neutropenic fever and hypotension. Empirical therapy with ceftazidime and gentamicin was initiated. Blood cultures revealed imipenem-resistant *Serratia marcescens* with retained sensitivity to meropenem and a range of other antibiotic agents (Table 1). On Day 20, gentamicin was replaced with ciprofloxacin owing to renal insufficiency and the patient completed a treatment course with no further positive cultures.

2.2. Case 2

A 3.5-year-old Caucasian male from Alaska was hospitalised for induction chemotherapy for newly diagnosed AML. His early hospital course was complicated by neutropenic fever that was treated empirically with ceftazidime. Antibiotic therapy was changed to meropenem after 6 days owing to a presumed drug rash and the patient continued on empirical meropenem for a total course of 23 days. He had no positive blood cultures during this time and had been afebrile for 11 days at the time of stopping antibiotics. A second course of induction chemotherapy was initiated on Day 28 owing to persistent evidence of leukaemic disease. On Day 32 he became febrile and meropenem was administered. On Day 42, a blood culture obtained from his central venous catheter grew carbapenem-resistant *Escherichia coli*; the pathogen remained susceptible to aminoglycosides. He was treated with gentamicin and no further positive cultures were recovered.

2.3. Case 3

An 11-year-old Hispanic male with relapsed acute lymphoblastic leukaemia (ALL) was admitted for severe nausea and vomiting of 5 days duration. Just prior to his admission he had received consolidation chemotherapy in Mexico. Upon hospitalisation, empirical therapy with ceftazidime, gentamicin and vancomycin was initiated. Cultures of blood and stool revealed carbapenem-resistant *E. coli*. Based on susceptibility results (Table 1), his treatment was changed to gentamicin and trimethoprim/sulfamethoxazole and he completed therapy with all subsequent cultures remaining negative.

2.4. Case 4

A 17-year-old Caucasian female from New Jersey was admitted for unrelated-donor haematopoietic cell transplantation for ALL. She had previously been hospitalised in New Jersey for chemotherapy and was transferred to SCH for transplantation evaluation after her second remission. On Day 5 post transplantation she developed neutropenic fevers and severe mucositis and was empirically treated with meropenem and gentamicin. On Day 9 post transplantation she became haemodynamically unstable. Gentamicin

Table 1
Resistance phenotype and minimum inhibitory concentrations (in mg/L) for carbapenem-resistant Enterobacteriaceae[a].

Case	Species	MER [≥4]	IPM [≥4]	DOR [≥4]	ERT [≥1]	CAZ [≥16]	TZP [≥128]	CIP [≥4]	GEN [≥16]	AMK [≥64]	TOB [≥16]	SXT [≥4/76]	SUL [≥512]	FOS [≥256]	COL [>2]	TIG [>4]	MHT
1	*Serratia marcescens*	S (1)	R (>32)	S (2)	R (2)	S	S	S	S	S	S	S	S	S (12)	R (>256)	S (4)	–
2	*Escherichia coli*	R (16)	R (32)	R (16)	R (>32)	R	R	S	S	S	S	S	R	S (6)	S (0.38)	S (0.125)	–
3	*E. coli*	R (16)	R (32)	R (16)	R (>32)	R	R	R	S	I	R	S	R	S (4)	S (0.125)	S (0.5)	–
4	*Klebsiella pneumoniae*	R (32)	R (32)	R (32)	R (32)	R	R	R	R	R	R	R	R	R (256)	S (0.5)	S (1)	+
5	*K. pneumoniae*	R (16)	S (1.5)	R (4)	R (32)	R	R	I	R	S	S	R	R	R (1024)	S (1)	S (1.5)	–
6	*K. pneumoniae*	S (1.5)	S (0.25)	S (1)	R (12)	R	R	R	R	S	S	R	R	S (64)	S (1)	S (0.75)	–

MER, meropenem; IPM, imipenem; DOR, doripenem; ERT, ertapenem; CAZ, ceftazidime; TZP, piperacillin/tazobactam; CIP, ciprofloxacin; GEN, gentamicin; AMK, amikacin; TOB, tobramycin; SXT, trimethoprim/sulfamethoxazole; SUL, sulfamethoxazole; FOS, fosfomycin; COL, colistin; TIG, tigecycline; MHT, Modified Hodge test; S, susceptible; I, intermediate; R, resistant.

[a] Reported interpretation (S, I and R) and minimum inhibitory concentration breakpoints (in brackets) are per Clinical and Laboratory Standards Institute (CLSI) document M100-S21, with the exception of colistin and tigecycline where European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2010 breakpoints were used.