Molecular and clinical epidemiology of extended-spectrum cephalosporin-resistant infections in children caused by *Enterobacteriaceae* species other than *E. coli* and *K. pneumoniae*

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Background

Resistance to extended-spectrum cephalosporins (ESC-R) in *Enterobacteriaceae* is a growing concern in children but little data has been reported for species other than *E. coli* and *K. pneumoniae*. We aimed to describe the clinical, microbiological, and molecular characteristics of ESC-R infections in children caused by *Enterobacteriaceae* species other than *E. coli* and *K. pneumoniae.*

Methods

Setting

- Prospective surveillance study at 4 freestanding US pediatric hospitals (**Figure 1**)

Figure 1: Geographic distribution of study sites

Subjects and Study isolates

- ESC-R *Enterobacteriaceae* isolates recovered from normally sterile sites (including stool for *Salmonella* and *Shigella*) of patients aged ≤ 21 years.
- *E. coli*, *Klebsiella* spp, *P. mirabilis*, *Salmonella* and *Shigella* isolates not susceptible to 3rd generation cephalosporins or cefepime, using the routine microbiology methods at each hospital, were defined as ESC-R.
- Isolates were sent to the coordinating center quarterly.

Clinical and Hospital Data

- Associated clinical data were obtained for all isolates via medical record review.
- The total isolates by species recovered from normally sterile sites (including stool for *Salmonella* and *Shigella*) during the study period at each hospital was used to estimate prevalence.

Laboratory Methods

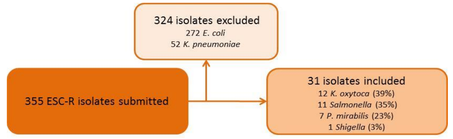
- Upon arrival, species were identified using Vitek 2 with Advanced Expert System.
- Isolates were tested for susceptibility to ampicillin, amoxicillin-clavulanic acid, cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, meropenem, piperacillin-tazobactam, ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole by disk diffusion using 2010 CLSI guidelines.
- Susceptibility phenotypes were further characterized using disks for cefpodoxime, cefepime, ceftazidime/ceftazidime-clavulanic acid, cefotaxime/cefotaxime-clavulanic acid, and Etests.
- All isolates were tested by PCR for *bla* genes encoding the most common extended-spectrum cephalosporinases (CTX-M, CMY, DHA, SHV and FOX).

Results

Study Isolates

- Between October 2009 and September 2013, 355 isolates met criteria for ESC-R; 91% were *E. coli* or *K. pneumoniae* and were excluded from this study (**Figure 2**)

Figure 2. Study isolates



- The prevalence of ESC-R among these species was 0.9% and did not significantly differ by study year (**Table 1**).

Table 1. Frequency of Isolates by Study Year

	Year 1	Year 2	Year 3	Year4
All species	8/907* (0.9%)	10/928 (1.1%)	7/1015 (0.7%)	6/789 (0.8%)
K. Oxytoca	1/176 (0.6%)	5/190 (2.6%)	4/211 (2.4%)	2/170 (1.2%)
P. Mirabilis	2/467 (0.9%)	2/521 (0.4%)	1/593 (0.2%)	2/434 (0.5%)
Salmonella	4/217 (1.8%)	3/174 (1.7%)	2/172 (1.2%)	2/170 (1.2%)
Shigella	1/47* (2%)	0/43 (0%)	0/39 (0%)	0/15 (0%)

* *Shigella* isolates from 2 study sites that were experiencing a community-wide outbreak are excluded (no resistant isolates were identified from these sites)..

Patient Characteristics (Table 2)

- Overall, the median age of patients was 3.8 years (range, 0.1-19.2) and 52% were male.
- All ESC-R *K. oxytoca* isolates were obtained from patients with underlying medical conditions and/or previous hospitalization, and 67% were hospital-acquired.
- ESC-R *P. mirabilis* isolates were most commonly obtained from the urine of patients with underlying urological conditions.
- As expected, ESC-R *Salmonella* and *Shigella* isolates were obtained from healthy children.

Table 2. Demographics and clinical characteristics

	K. oxytoca n=12	*P. mirabilis* n=7	*Salmonella* n=11	*Shigella* n=1
Hospital				
Seattle	3 (25%)	3 (43%)	6 (55%)	1 (100%)
Kansas City	3 (25%)	1 (14%)	1 (9%)	0 (0%)
St. Louis	3 (25%)	0 (0%)	0 (0%)	0 (0%)
Philadelphia	3 (25%)	3 (43%)	4 (36%)	0 (0%)
Median age, years (range)	4.8 (0.4-18.5)	3.8 (1.9-19.2)	0.9 (0.1-15.4)	18
IQR	2.1, 11.7	2.9, 8.8	0.2, 3.8	
Female gender	7 (58%)	3 (43%)	4 (36%)	1 (100%)
Hispanic Ethnicity	1 (8%)	0 (0%)	3 (27%)	(0%)
Non-white Race	4 (33%)	4 (33%)	5 (45%)	1 (100%)
Site of Culture				
Urine	7 (58%)	6 (86%)	0 (0%)	0 (0%)
Blood	2 (17%)	1 (14%)	0 (0%)	0 (0%)
Stool	0 (0%)	0 (0%)	10 (91%)	1 (100%)
Other	3 (25%)	0 (0%)	1 (9%)	0 (0%)
Onset				
Community-associated	0 (0%)	2 (29%)	8 (73%)	1 (100%)
Healthcare-associated	4 (33%)	4 (57%)	2 (18%)	0 (0%)
Hospital-acquired	8 (67%)	1 (14%)	1 (9%)	0 (0%)
Hospitalized (in last year)	5 (42%)	3 (43%)	2 (18%)	0 (0%)
Medical Condition				
Neurological	2 (17%)	3 (43%)	1 (9%)	0 (0%)
Cardiovascular	3 (25%)	2 (29%)	0 (0%)	0 (0%)
Urological	3 (25%)	3 (43%)	1 (9%)	0 (0%)
Other	6 (50%)	4 (57%)	1 (9%)	0 (0%)
History of Transplantation	3 (25%)	1 (14%)	0 (0%)	0 (0%)
Immunosuppressants	9 (75%)	1 (14%)	0 (0%)	0 (0%)
Indwelling device	11 (92%)	3 (43%)	1 (9%)	0 (0%)

Bacterial Characteristics (Table 3)

- The ESBL phenotype was present in 13 (42%) isolates, the AmpC phenotype was present in 13 (42%) isolates, and 5 (16%) isolates did not exhibit a characteristic ESC-R phenotype despite non-susceptibility to ESCs.
- An ESBL determinant was detected in 6 (46%) isolates with an ESBL phenotype (CTX-M =4 and SHV = 2).
- An AmpC determinant was detected in all isolates with an AmpC phenotype (CMY =12 and DHA n=1).
- Of interest, 9 *Salmonella* isolates carried CMY-2.

Table 3. Microbiological and molecular Characteristics.

				ESC-R Determinant					
				Any Detected		Determinants Detected			
	#	Phenotype	#	No	Yes	CTX-M	SHV	CMY	DHA
K. oxytoca	12	ESBL	9	7	2	0	2	0	0
		None	3	3	0	0	0	0	0
P. mirabilis	7	ESBL	1	0	1	1	0	0	0
		AmpC	4	0	4	0	0	3	1
		None	2	0	2	0	0	2	0
Salmonella	11	ESBL	2	0	2	2	0	0	0
		AmpC	9	0	9	0	0	9	0
Shigella	1	ESBL	1	0	1	1	0	0	0

Co-resistance (Table 4)

- Overall, resistance to trimethoprim-sulfamethoxazole (TMP/SMX), gentamicin, and ciprofloxacin occurred in 32%, 23%, and 6% of these ESC-R isolates, respectively.
- Resistance to TMP/SMX was the highest among *K. oxytoca* isolates.

Table 4. Non-susceptibility to common non-beta-lactams.

	K. Oxytoca n=12	*P. Mirabilis* n=7	*Salmonella* n=11	*Shigella* n=1
Non-susceptible to:				
TMP/SMX	5 (42%)	1 (14%)	3 (27%)	1 (100%)
Ciprofloxacin	1 (8%)	1 (14%)	0 (0%)	0 (0%)
Gentamicin	2 (17%)	2 (28%)	3 (27%)	0 (0%)
TMP/SMX and Cipro	1 (8%)	1 (14%)	0 (0%)	0 (0%)

Conclusions

- Overall, 9% of ESC-R infections in children were caused by *Enterobacteriaceae* species other than *E. coli* and *K. pneumoniae.*
- ESBL or AmpC phenotypes and determinants were common among these isolates.
- TMP/SMX resistance occurred in 32% of isolates, while ciprofloxacin resistance was uncommon.