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Association of systemic antimicrobials with the expression of beta-lactamases in bacteria cultured from urological patients

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ABSTRACT

Background: Patients with abnormalities of the genitourinary tract are at high risk for infections with antimicrobial-resistant pathogens.
Methods: All urine cultures ordered by members of the Division of Urology from four quarterly one-week periods were included. All gram-negative bacilli isolated were analyzed using the Check-Points Check-MDR CT103XL assay to identify the presence of genes associated with resistance to beta-lactam antibiotics. Association between the days of antibiotics and the presence of an ESBL-producing organism was determined.
Results: One hundred eleven positive cultures were included in this analysis, of which 5 (4.5%) contained ESBL-producing species. Days of systemic antibiotics within 30 days of urine culture was associated with an increased risk of isolating an ESBL-producing pathogen.
Conclusion: The overall prevalence of ESBL-producing organisms is low in this cohort. The number of days of systemic antibiotics within 30 days of a urine culture was significantly associated with increased risk of isolating an ESBL-producing organism.

1. Introduction

Infection due to antimicrobial-resistant bacteria is a public health threat (Frieden, 2013). Currently, many microbiology laboratories have the capability to test for some common resistance genes (*mecA, vanA*, select carbapenemase genes) but lack the ability to detect common antimicrobial resistance genes in Gram-negative rods. Rapid and comprehensive recognition of antimicrobial resistance is essential in order to initiate appropriate antimicrobial therapy. Identification of genes associated with resistance to third-generation cephalosporins – extended spectrum beta-lactamases (ESBLs) and AmpC genes – at the time of pathogen identification would allow for appropriate antimicrobial selection and potentially prevent clinical decompensation due to insufficient antimicrobial coverage. To maximize the impact of antimicrobial stewardship, it is critical to understand antimicrobial resistance profiles in key groups of high-risk patients. Patients with abnormalities of the genitourinary (GU) tract are at high risk for infections with antimicrobial -resistant organisms (Forster et al., 2016), although the exact prevalence of specific genes of resistance in pediatric urologic patients is unknown.

Patients with anatomic abnormalities of the GU tract – especially those with neurogenic bladders - frequently receive antimicrobial agents for both the prevention and treatment of bacteriuria (Routh et al., 2016). These agents may be systemic or intravesical – administered directly into the bladder via catheter. While it has been shown that systemic antimicrobial administration is a known factor in the development of antimicrobial resistance (Zegers et al., 2017), there is less data regarding the association with intravesical antimicrobials and resistance. While intravesical antimicrobials are used frequently within our Division of Urology as UTI prophylaxis for children who require clean intermittent catheterization (CIC), this is not a universal practice. Therefore, this allows a unique opportunity to investigate the effects of both intravesical and systemic antimicrobials versus systemic antimicrobials alone.

The Check-Points multiplex PCR is a molecular diagnostic test that allows for the detection of approximately 150 genes associated with resistance to beta-lactam antimicrobials. We sought to determine the prevalence of genes associated with resistance to third-generation cephalosporins in Gram-negative bacilli isolated from urological

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patients, and determine the association between presence of organisms having these resistance genes and recent receipt of either systemic or intravesical antimicrobials.

2. Methods

Quarterly, all positive urine cultures ordered by clinicians in the Division of Urology for 1 week (November 12–19, 2015; February 10–17, 2016; May 12–19, 2016; and August 10–17, 2016) within a 1-year period were included in this study.

Urine samples were inoculated onto BBL 5% sheep blood agar and MacConkey agar (Becton, Dickinson, and Company, Franklin Lakes, NJ, USA) and incubated at 35 °C ambient air for 18–24 hours. Gram-negative bacilli from positive cultures were frozen in Brucella broth with glycerol at −80 °C. Once all of the organisms were collected, the isolates were subcultured onto 5% sheep blood agar and DNA was extracted with the bioMérieux nucliSENS easyMAG nucleic acid extractor (bioMérieux, Marcy l'Etoile, France) (Powell and Mortensen, 2016).

The Check-Points Check-MDR CT103XL assay was performed according to manufacturer recommendations (Check-Points BV, Wageningen, Netherlands). Briefly, beta-lactamase genes are amplified in a multiplex PCR. The resulting products are detected with a tube microarray (Bogaerts et al., 2016).

Based on the genes detected by the Check-Points system, isolates were grouped together into the following groups: negative, TEM WT, SHV WT, AmpC, ESBL or CRE. Isolates with either CMYII or ACT/MIR either alone or in combination with TEM WT were included in the AmpC group. Bacterial isolates that had the presence of CTX-M-1 or CTX-M-9, either with or without the presence of TEM WT, were included in the ESBL group.

All clinical data were collected from the electronic health record through structured chart review. Antimicrobial exposure data was obtained from a database maintained by the Antimicrobial Stewardship Program. Duration of antimicrobial therapy was confirmed by manual chart review. The Institutional Review Board at Cincinnati Children's Hospital Medical Center approved this study.

Descriptive statistics were used to determine the point prevalence of specific genotypes of resistance during these specified time periods. Categorical data were compared between groups using chi-square, or fisher's exact as warranted. ANOVA and post-hoc Tukey were used to compare continuous variables between groups. Logistic regression was used to determine the association between days of antimicrobials and the presence of a specific genotype of resistance. Given the small number of patients with organisms with ESBL, AmpC, or SHV WT, only one covariate was included in the model.

3. Results

The mean of age of the patients included in study was 12.3 (standard deviation 12.6) years. There was no difference in mean age between patients in any of the groups. There were fewer females in the AmpC group compared to the negative, TEM WT, and SHV WT group. There was no difference in the proportion of patients who require CIC between the groups, although there was a higher proportion of patients in both the SHV WT and AmpC groups who had an external catheterizable conduit compared to both the negative and SHV WT groups.

During the time periods studied, 111 cultures grew a Gram-negative bacillus. 61 of the pathogens were negative for genes of resistance, 40 had narrow-spectrum beta-lactamases (wild-type TEM or SHV), and 10 had genes associated with resistance to third-generation cephalosporins (5 Amp. including 4 ACT/MIR and 1 CMYII, 5 ESBL including 4 CTX-M-1 and 1 CTX-M-9) (Table 1). *Escherichia coli* was the most common pathogen, accounting for 68.5% of the total cultures, followed by *Klebsiella pneumoniae* (6.3%), *Pseudomonas aeruginosa, Proteus mirabilis* (5.4% each), and *Enterobacter* species (4.5%). No carbapenemase genes were detected.

There was no difference in the number of days of total (systemic and intravesical) antimicrobials at 30, 60, 90, 180, or 360 days prior to urine culture between groups. The most frequently received antimicrobial was sulfamethoxazole-trimethoprim (n = 37 patients in the 360 days preceding urine culture), followed by amoxicillin (n = 21), intravesical gentamicin (n = 19), cefdinir (n = 17) and cephalexin (n = 17) (Supplemental Table 1). There were significantly more days of systemic antimicrobials within 30 days of urine culture in the ESBL group compared to the negative group (11.2 ± 13.2, 2.0 ± 5.8, p = 0.05) (Table 2). Logistic regression for the outcome of an ESBL-producing organism in urine culture shows an odds ratio of 1.09 (1.01–1.17) for number of days of systemic antimicrobials administered within the 30 days prior to urine culture.

4. Discussion

In this study, we report the prevalence of antimicrobial resistance genes in Gram-negative rods isolated from pediatric urology patients. We also report an increased risk of having an ESBL-producing organism in urine culture with increased number of days of systemic antimicrobial in the 30 days leading up to the culture.

The prevalence of ESBL-producing organisms reported here is higher than has been reported in the general pediatric population (Fan et al., 2014; Hiyama et al., 2015). However, many studies that report prevalence rates of ESBL-producing organisms define ESBL phenotypically based on the CLSI guidelines. Additionally, much of the work in the

Table 1
Beta-Lactamases detected by species.

Isolate	Negative	TEM WT	SHV WT	AmpC	ESBL
Escherichia coli	39 (63.9%)[b]	33 (94.3%)	0[a,b]	0[a,b]	4 (80%)[c,d]
Klebsiella pneumoniae	1 (1.6%)	0	5 (100%)[a,b,d,e]	0	1 (20%)
Pseudomonas aeruginosa	6 (9.8%)	0	0	0	0
Proteus mirabilis	6 (9.8%)	0	0	0	0
Enterobacter species	1 (1.6%)	0	0	4 (80%)[a,b,c,e]	0
Citrobacter freundii	1 (1.6%)	2 (5.7%)	0	0	0
Klebsiella oxytoca	2 (3.3%)	0	0	0	0
Citrobacter koseri	1 (1.6%)	0	0	0	0
Citrobacter species	0	0	0	1 (20%)	0
Enterobacter aerogenes	1 (1.6%)	0	0	0	0
Providencia rettgeri	1 (1.6%)	0	0	0	0
Pantoea species	1 (1.6%)	0	0	0	0
Serratia marcescens	1 (1.6%)	0	0	0	0

[a] $P < 0.05$ compared to Negative.
[b] $P < 0.05$ compared to TEM WT.
[c] $P < 0.05$ compared to SHV WT.
[d] $P < 0.05$ compared to AmpC.
[e] $P < 0.05$ compared to ESBL.

Table 2
Days of antimicrobial therapy.

	Negative (n = 61)	TEM WT (n = 35)	SHV WT (n = 5)	AmpC (n = 5)	ESBL (n = 5)
Days of all antibiotics (systemic and intravesical)					
Previous year	71.9 (119.4)	98.9 (152.5)	64.8 (80.1)	84.2 (95.4)	142.4 (150.2)
Previous 180 days	33.6 (59.6)	42.5 (73.9)	24.2 (19.5)	19.0 (27.3)	44.6 (54.8)
Previous 90 days	11.5 (25.2)	13.4 (30.0)	20.0 (20.3)	1.2 (2.7)	28.2 (40.1)
Previous 60 days	8.2 (19.7)	10.1 (23.4)	7.4 (11.8)	0 (0)	18.6 (28.8)
Previous 30 days	4.0 (10.4)	4.3 (12.4)	1.8 (4.0)	0 (0)	11.2 (13.2)
Days of systemic antibiotics					
Previous year	46.7 (82.8)	58.5 (88.7)	64.8 (80.1)	84.2 (95.4)	69.4 (92.6)
Previous 180 days	16.7 (26.5)	20.5 (37.2)	24.2 (19.5)	19.0 (27.3)	44.6 (54.8)
Previous 90 days	8.5 (16.1)	11.7 (22.8)	20.0 (20.3)	1.2 (2.7)	28.2 (40.1)
Previous 60 days	4.8 (10.8)	8.4 (16.7)	7.4 (11.8)	0 (0)	18.6 (28.8)
Previous 30 days	2 (5.8)	3.4 (9.0)	1.8 (4.0)	0 (0)	11.2 (13.2)[a]

[a] $P = 0.05$ compared to negative.

literature focuses on the presence of CTX-M genes in specific bacterial species rather than all pathogens that have the potential to acquire this gene. Therefore, our results obtained using the definition of ESBL based on the presence of causative genes (e.g. CTX-M) and across all potential ESBL-producing isolates are not directly comparable to prior studies. Additionally, AmpC genes have not been included in previous studies. It is critical to recognize AmpC producing organisms even when the gene is not induced, as third-generation cephalosporin use may lead to gene induction and treatment failure.

The association between antimicrobial receipt and UTI with antimicrobial resistant organism has been previously reported, though no previous studies have included intravesical antimicrobials (Hertz et al., 2016; Inns et al., 2014; Paschke et al., 2010). In this study, there was an association between infection with an ESBL-producing organism and more days of systemic antimicrobials in the previous 30 days. This association was not found when intravesical antimicrobials were also included in the analysis. This may be due to their differential effect on the microbiome. The gastrointestinal (GI) tract is the source of many of the multidrug resistant strains responsible for infection (Manges et al., 2016). Systemic antimicrobial administration alters the GI microbiota, and is associated with increased risk of infections with multidrug resistant organisms (Bell et al., 2014). Further, administration of systemic antimicrobials is associated with increased susceptibility to systemic infections (Brown and Clarke, 2017). However, this effect is temporary as systemic antimicrobials have been shown to have significant effects on the GI microbiome that begins to normalize within 4 weeks following antimicrobial discontinuation (Dethlefsen et al., 2008). There is limited data on the effects of intravesical antimicrobials on the microbiome, but given its lack of systemic absorption, it likely has much less of an effect on the GI microbiome, and therefore, the development of infections with ESBL-producing organisms.

This study is not without limitations. Due to inclusion criteria, no patients without bacteriuria were included in this study. This study represents results from a single center without longitudinal data. Additionally, the effects of intravesical antimicrobials cannot be isolated, as patients received both systemic and intravesical antimicrobials in combination. To more fully explore the effects of intravesical antimicrobials, future work will focus on larger cohorts with longitudinal data.

5. Conclusion

Within a subset of all patients followed by the Division of Urology at Cincinnati Children's Hospital Medical Center, the prevalence of isolates with genes associated with resistance to third-generation cephalosporins is 9.0%. The prevalence of any genotypic resistance (including narrow-spectrum penicillinases) is 45.1%. The number of days of systemic antimicrobials within 30 days prior to culture is significantly associated with the presence of an ESBL-producing isolate cultured from urine. Antimicrobial stewardship and improved methods to diagnose, and therefore treat, UTIs are needed to prevent increasing rates of antimicrobial resistance.

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IRB oversight

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