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## Antimicrobial Drug–Resistant Shiga Toxin–Producing *Escherichia coli* Infections, Michigan, USA

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High frequencies of antimicrobial drug resistance were observed in O157 and non-O157 Shiga toxin–producing *E. coli* strains recovered from patients in Michigan during 2010–2014. Resistance was more common in non-O157 strains and independently associated with hospitalization, indicating that resistance could contribute to more severe disease outcomes.

Shiga toxin–producing *Escherichia coli* (STEC) contributes to 265,000 cases of foodborne illness annually

in the United States (1). Most infections are caused by O157 strains; however, non-O157 STEC infections have increased (2). Antimicrobial drug resistance among STEC has been reported (3–5) but is probably underestimated. Given the importance of resistance in *E. coli* pathotypes, we sought to determine the prevalence of resistant STEC infections and assess the effects of resistance on disease.

We obtained 358 STEC isolates from the Michigan Department of Health and Human Services (MDHHS) Reference Laboratory (Lansing, MI, USA), collected during 2010–2014. Of these, 14 were outbreak associated. We examined 1 strain per outbreak using protocols approved by Michigan State University (MSU; Lansing, MI, USA; IRB #10-736SM) and MDHHS (842-PHALAB). Overall, 31 (8.8%) strains (23 non-O157, 8 O157) were resistant to antimicrobial drugs (Table). Resistance to ampicillin (7.4%) was most common, followed by trimethoprim/sulfamethoxazole (SXT) (4.0%) and ciprofloxacin (0.3%). Compared with national rates, resistance to ampicillin and SXT was higher, but not significantly different, for O157 isolates from Michigan (online Technical Appendix Figure 1, <https://wwwnc.cdc.gov/EID/article/23/9/17-0523-Techapp1.pdf>) (6). One strain was resistant to all drugs, and all resistant strains had high MICs (ampicillin, >64 µg/mL; ciprofloxacin, >32 µg/mL; SXT, in 1:19 ratio, >32/608 µg/mL). Notably, resistance was twice as common for non-O157 (11.1%) than for O157 (5.5%) strains. O111 strains (n = 7) had significantly higher resistance frequencies (24.1%) than other non-O157 serogroups (p = 0.03). We found variation by year and season; resistance frequencies were highest in 2012 (online Technical Appendix, Figure 2) and during winter/spring (online Technical Appendix Table 1), but neither trend was significant. We also observed a strong but nonsignificant association between resistance and hospitalization but no association for urban versus rural residence (7) or county after stratifying by prescription rates (8) in the univariate analyses.

We conducted a multivariate analysis using logistic regression, with hospitalization as the dependent variable; we included variables with significant (p < 0.05) and strong (p < 0.20) associations from the univariate analysis as independent variables. Forward selection indicated that hospitalized patients were more likely to have resistant infections (odds ratio [OR] 2.4, 95% CI 1.00–5.82) and less likely to have non-O157 infections (OR 0.4, 95% CI 0.21–0.61) (online Technical Appendix Table 2), suggesting that resistant infections or O157 infections may cause more severe clinical outcomes. Patients ≥18 years of age, women, and patients with bloody diarrhea were also more likely to be hospitalized.

Although we found no significant difference by *stx* profile, strains possessing *stx1* only were more commonly resistant than strains with *stx2* alone (p = 0.27 by Fisher exact test). All 23 (100%) resistant non-O157 STEC and 1

**Table.** Antimicrobial drug resistance in 353 clinical Shiga toxin-producing *Escherichia coli* isolates, by serotype, Michigan, USA, 2010–2014\*

Serotype	No. isolates	No. (%) isolates			
		Any resistance	Ampicillin resistance	Ciprofloxacin resistance	SXT resistance
O157	146	8 (5.5)	7 (4.8)	0 (0)	5 (3.4)
Non-O157	207	23 (11.1)	19 (9.2)	1 (0.5)	9 (4.3)
O26	53	4 (7.6)	4 (7.6)	0 (0)	1 (1.9)
O45	50	6 (12.0)	5 (10.0)	0 (0)	2 (4.0)
O103	75	6 (8.0)	5 (6.7)	1 (1.3)	4 (5.3)
O111	29	7 (24.1)	5 (17.2)	0 (0)	2 (6.9)

\*We tested 358 isolates by disk diffusion for resistance to ampicillin (10 µg in disk), SXT (25 µg in disk), and ciprofloxacin (5 µg in disk). MICs were determined by using Etest. Strains were classified as resistant or susceptible according to Clinical Laboratory Standards Institute guidelines; *E. coli* ATCC 25922 was used as a control. Five isolates had unknown serotypes and were excluded from analysis. Isolate numbers for individual antibiotics do not always add up to the total number of isolates with any resistance because some isolates were resistant to >1 drug. SXT, trimethoprim/sulfamethoxazole.

(12.5%) resistant O157 strain had *stx1* only. Strains positive for *eae* were less likely to be resistant ( $n = 27$ ; 8.4%) than *eae*-negative strains ( $n = 4$ ; 23.5%); this nonsignificant difference ( $p = 0.07$  by Fisher exact test) could be due to small sample sizes. All 8 resistant O157 strains and 18 (78.3%) of 23 resistant non-O157 strains had *eae*, demonstrating correlations between virulence genes and serogroups.

Overall, we detected a high frequency of resistance among non-O157 STEC (11.2%), similar to findings from Mexico (15%), although we evaluated fewer drugs (5). Resistance to ciprofloxacin was low despite its routine use for treating enteric infections, perhaps because resistance development in *E. coli* requires multiple mutations (9). Resistance frequencies in STEC were low relative to other *E. coli* pathotypes such as extraintestinal *E. coli*, which may be attributable to differences in the source of the infections (3).

The higher O157 resistance frequencies in Michigan than nationwide indicate that selection pressures vary by location and source. Although we observed no difference in resistance frequencies for counties with high versus low antimicrobial drug prescription rates (8), we have not investigated selection pressures from drug use in farm environments that may affect resistance emergence in Michigan. Approximately  $12 \times 10^6$  kg of antimicrobial drugs are administered to food animals annually in the United States; roughly 61% of these are medically relevant. Higher resistance frequencies in winter/spring (12.2%) than summer/fall (7.5%) could be attributed to variation in prescription rates by season (10).

Because Michigan is not part of the Centers for Disease Control and Prevention Foodborne Diseases Active Surveillance Network and resistance in STEC has not been widely researched, data about the prevalence and impact of resistance are lacking. This study detected a high frequency of STEC resistance to antimicrobial drugs commonly used in human and veterinary medicine, particularly for non-O157 serotypes, which have increased in frequency (2). Monitoring resistance in STEC is essential because of the risk of transmitting resistant strains from food animals to humans and the high likelihood of horizontal transfer of resistance

genes from STEC to other pathogens. Routine monitoring can uncover new treatment approaches and guide development of strategies for controlling emergence and spread of resistance in STEC and other *E. coli* pathotypes.

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## White-Nose Syndrome Fungus in a 1918 Bat Specimen from France

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White-nose syndrome, first diagnosed in North America in 2006, causes mass deaths among bats in North America. We found the causative fungus, *Pseudogymnoascus destructans*, in a 1918 sample collected in Europe, where bats have now adapted to the fungus. These results are consistent with a Eurasian origin of the pathogen.

We report the earliest known historical incidence of the fungus *Pseudogymnoascus* (formerly *Geomyces*) *destructans*, detected in a museum specimen of a bat (*Myotis bechsteinii*) collected in France in 1918. This

fungal pathogen causes white-nose syndrome (WNS) in bats (*I*). Since its introduction into eastern North America around 2006, WNS has devastated bat populations across the continent (*2*). *P. destructans* has also been found across the Eurasian landmass (*3,4*) without documented mass bat deaths. Epidemiologic evidence among bats and fungal genetics indicate that the fungus has been recently introduced into North American bat populations (*5–7*).

To clarify the epidemiologic history of WNS and to investigate physical evidence of its presence in specific locations in the past, we screened 138 19th- and 20th-century bat specimens (housed at the National Museum of Natural History [USNM], Washington, DC) from North America (n = 41), Europe (n = 83), and East Asia (n = 14) for *P. destructans* DNA (online Technical Appendix, <https://wwwnc.cdc.gov/EID/article/23/9/17-0875-Techapp1.pdf>). We sampled dry museum skins and intact bodies stored in 70% ethanol; some were originally fixed in formalin. We swabbed bat rostra and wings to collect potentially preserved *P. destructans* biomolecules and stored swabs in 100% ethanol until DNA extraction.

We extracted DNA in a dedicated ancient DNA laboratory at the National Zoological Park (Washington, DC) by using stringent protocols to prevent false positive results from modern DNA contamination (*8*). Before extraction, we removed swabs from the ethanol and let them air dry. We then let swabs digest overnight at 55°C in 600 µL extraction buffer (1× Tris-EDTA buffer, pH 8.0, 0.019 mmol/L EDTA, 0.01 mmol/L NaCl, 1% SDS, 10 mg/mL DTT, and 1 mg/mL proteinase K) (*8*). Later extractions omitted DTT. We extracted digested samples twice in 600 µL phenol and once in 600 µL chloroform. We removed and concentrated the aqueous phase by using Amicon Ultra-4 30 kDA molecular weight cutoff columns (Millipore Sigma, Merck, Billerica, MA, USA) to a final volume of ≈250 µL. We included 1 extraction blank for every 10–11 historical samples.

We screened extracts for *P. destructans* by using a previously described species-specific quantitative PCR targeting 103 bp (including primers) of the intergenic spacer region (*9*). Each extract was amplified in 2–8 replicate PCRs. Multiple, no-template controls (*2,3*) were included in each PCR setup. Positive products from experiments in which quantifiable contamination (>0.1 genome equivalents/µL sample) was observed in ≥1 negative control were discarded; these experiments were repeated with fresh reagents.

One sample (USNM 231170) tested positive in 2 of 3 PCRs. We performed a second independent extraction on this sample. The replicate extraction tested positive in 4 of 5 PCRs. Two of the USNM 231170-positive PCR products were confirmed by using Sanger sequencing and comparison to publicly available *P. destructans* sequences in GenBank. These sequences were 100% identical to *P. destructans* sequences from North America (GenBank accession nos. JX270192.1