We carried out a molecular biological analysis of extended-spectrum β-lactamase (ESBL)-producing *E. coli* strains and their sensitivity to flomoxef (FMOX). Sequence type (ST) analysis by multilocus sequence typing (MLST) and classification of ESBL genotypes by multiplex PCR were performed on ESBL-producing *E. coli* strains isolated from urine samples collected from patients treated at our institution between 2008 and 2018. These sequences were compared with results for antimicrobial drug susceptibility determined using a micro-liquid dilution method. We also analyzed cases treated with FMOX at our institution to examine its clinical efficacy. Of the 911 *E. coli* strains identified, 158 (17.3%) were ESBL-producing. Of these, 67.7% (107/158) were strain ST-131 in ST analysis. Nearly all (154/158; 97.5%) were CTX-M genotypes, with M-14 and M-27 predominating. The isolated strains were sensitive to FMOX in drug susceptibility tests. Among the patient samples, 33 cases received FMOX, and of these, 5 had ESBL-producing *E. coli*. Among these five cases, three received FMOX for surgical prophylaxis as urinary carriers of ESBL-producing *E. coli*, and postoperative infections were prevented in all three patients. The other two patients received FMOX treatment for urinary tract infections. FMOX treatment was successful for one, and the other was switched to carbapenem. Our results suggest that FMOX has efficacy for perioperative prophylactic administration in urologic surgery involving carriers of ESBL-producing bacteria and for therapeutic administration for urinary tract infections. Use of FMOX avoids over-reliance on carbapenems or β-lactamase inhibitors and thus is an effective antimicrobial countermeasure.