To evaluate a genome-based surveillance of all Danish third-generation cephalosporin-resistant Escherichia coli (3GC-R Ec) from bloodstream infections between 2014 and 2015, focusing on horizontally transferable resistance mechanisms. A collection of 552 3GC-R Ec isolates were whole-genome sequenced and characterized by using the batch uploader from the Center for Genomic Epidemiology (CGE) and automatically analysed using the CGE tools according to resistance profile, MLST, serotype and fimH subtype. Additionally, the phylogenetic relationship of the isolates was analysed by SNP analysis. The majority of the 552 isolates were ESBL producers (89%), with bla CTX-M-15 being the most prevalent (50%) gene, followed by bla CTX-M-14 (14%), bla CTX-M-27 (11%) and bla CTX-M-101 (5%). ST131 was detected in 50% of the E. coli isolates, with the remaining isolates belonging to 73 other STs, including globally disseminated STs (e.g. ST10, ST38, ST58, ST69 and ST410). Five of the bloodstream isolates were carbapenemase producers, carrying bla OXA-181 (3) and bla OXA-48 (2). Phylogenetic analysis revealed 15 possible national outbreaks during the 2-year period, one caused by a novel ST131/bla CTX-M-101 clone, here observed for the first time in Denmark. Additionally, the analysis revealed three individual cases with possible persistence of closely related clones collected more than 13 months apart. Continuous WGS-based national surveillance of 3GC-R Ec, in combination with more detailed epidemiological information, can improve the ability to follow the population dynamics of 3GC-R Ec, thus allowing for the detection of potential outbreaks and the effects of changing treatment regimens in the future.