mutations and at least one mutation in \textit{parC}, except for one strain with no \textit{parC} mutations. This is consistent with previous findings that \textit{gyrA} mutations are necessary for quinolone resistance, whereas \textit{parC} mutations appear to be complementary, as resistant strains do not carry \textit{parC} mutations alone.\textsuperscript{3–6} Some studies further suggest that additional \textit{parC} mutations facilitate increasing levels of resistance,\textsuperscript{5} but our study did not support this. For example, one isolate with an MIC of 4 mg/L had two \textit{gyrA} mutations only, whereas another isolate with an MIC of 1 mg/L had two \textit{gyrA} mutations and one \textit{parC} mutation. Other studies have also found no correlation between increasing QRDR mutations and MICS, suggesting that, in some populations, other mechanisms contribute towards quinolone resistance.\textsuperscript{6}

One isolate had a novel mutation in \textit{gyrA} (Ala-92 → Ser), along with mutations at positions 91 and 95. Isolates with triple \textit{gyrA} mutations have been rarely described.\textsuperscript{1} Previous reports of mutation at position 92 in \textit{gyrA} have resulted in a different substituted amino acid (Ala-92 → Pro).\textsuperscript{1} With the increasing use of non-culture molecular methods for the diagnosis of gonorrhoea, detailed knowledge of the range of mutations seen globally is essential to accurately detect antimicrobial resistance.

The commonest combination of mutations, seen in 11 (61\%) strains, involved two mutations in \textit{gyrA} (Ser-91 → Phe and Asp-95 → Gly) and one mutation in \textit{parC} (Asp-86 → Asn); of these, 9 had an additional silent mutation at codon 131 of \textit{parC}. Reported patterns of QRDR mutations vary between countries. This pattern of mutation positions, involving \textit{gyrA} positions 91 and 95 and \textit{parC} position 86, was also predominant in Austria, Philippines, Thailand, Denmark and Hawaii, but was not seen in India and Japan.\textsuperscript{4} The geographic variations may be due to clonal or polyclonal spread of different local or imported strains.\textsuperscript{5,6} Typing methods such as PFGE may help elucidate the molecular epidemiology and transmission networks of quinolone-resistant \textit{N. gonorrhoeae}.

In conclusion, quinolone-resistant \textit{N. gonorrhoeae} at our hospital have mutation patterns similar to some countries yet distinct from others and demonstrate no correlation between MICS and number of mutations. We also describe a novel \textit{gyrA} mutation. Although limited by the small number of isolates, this study enhances the existing knowledge of quinolone-resistant \textit{N. gonorrhoeae}.

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First detection of plasmid-mediated quinolone resistance (\textit{qnrA} and \textit{qnrS}) in \textit{Escherichia coli} strains isolated from humans in Scandinavia

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Sir,

Plasmid-mediated quinolone resistance linked to \textit{qnr} genes (\textit{A}, \textit{B} and \textit{S}) has recently been discovered.\textsuperscript{1–4} These encode Qnr proteins that are members of the pentapeptide family and are able to protect topoisomerases and thus reduce their susceptibility to fluoroquinolones and increase the mutant selection window, therefore increasing the likelihood of selection of mutations.\textsuperscript{4,5} Plasmid-mediated fluoroquinolone resistance associated with \textit{qnr} genes was first detected in the USA in 1994 in an isolate of \textit{Klebsiella pneumoniae}; later it has been found also in Asia and in several countries in Europe.\textsuperscript{4,5} These resistance determinants
might be a threat, allowing fast spread of resistance. Besides, qnr genes are often linked to resistance to cephalosporins.4,6 During a study that was performed to screen an Escherichia coli strain collection including 83 nalidixic-acid-resistant and 5 susceptible isolates from humans and 39 nalidixic-acid-resistant and 3 susceptible isolates from pigs in Denmark for characterization of quinolone resistance mechanisms, we found two isolates that showed reduced susceptibility to ciprofloxacin (MICs = 0.5 mg/L), but were susceptible to nalidixic acid (MICs = 4 and 8 mg/L). Both isolates were from patients at Hvidovre Hospital, Denmark: one from a urine sample and one from a blood sample.

In the first case, the patient was an 88-year-old woman hospitalized in mid-December 2005 with recurrent pneumonia, sepsis and bilateral crural ulcers. She was successfully treated with first mecillinam orally, then later cefuroxime and gentamicin iv and finally dicloxacillin orally due to a positive culture of Staphylococcus aureus from her crural ulcers. From a urine specimen, an E. coli resistant to ampicillin, cefuroxime, gentamicin, trimethoprim, sulfamethoxazole, ciprofloxacin and nitrofurantoin was cultured. This isolate, which showed no difference detected by antibiogram to the isolate later found, was, however, not further investigated or kept. In January 2006, the patient was hospitalized with pneumonia and dysuria and from a urine culture, 10⁵ cfu/mL of an extended-spectrum (ESBL)-producing E. coli was isolated (H88). This isolate showed the same susceptibility pattern as the first isolate at the hospital laboratory. By re-testing at the central lab, it was also considered resistant towards ceftriaxone, cefpodoxime and cefotaxime, but was ciprofloxacin-susceptible. The patient was treated successfully (clinically) with mecillinam 400 mg three times a day for 5 days.

In the second case, the patient was a 72-year-old male with disseminated colon cancer. His recent medical history included three abdominal operations and during the last month, he had received several antibiotics: ampicillin, gentamicin, metronidazole and meropenem. In April 2002, he showed clinical sepsis and an ESBL-producing E. coli was cultured from the blood (H93). This isolate was initially found to be resistant towards ampicillin, cefuroxime, ceftazidime and cefpodoxime, but intermediate to ceftriaxone and susceptible towards cefotaxin, cefotaxime, piperacillin + tazobactam and ciprofloxacin. The patient was initiated on iv piperacillin + tazobactam and ciprofloxacin, on which he improved clinically and became afebrile. During the subsequent 6 months, he deteriorated and finally succumbed to his cancer disease.

There were no indications in either case of acquisition of the strains outside Denmark. Previous to the current investigation, both E. coli strains were classified as ESBL producers. H88 harboured genes for the CTX-M-9 and TEM-1B β-lactamases and was, in this study, found to be resistant to ampicillin, cefalotin, cefpodoxime, ceftiofur, gentamicin, streptomycin, tetracyclines, sulfamethoxazole and trimethoprim. It was positive by PCR for aac(3')-IId, strA, strB, sul2 and tet(A) genes. Strain H93 harboured genes for the SHV-12 and TEM-1B β-lactamases and was, in this study, found to be resistant to ampicillin, cefalotin, cefpodoxime, chloramphenicol, spectinomycin, gentamicin, streptomycin, sulfamethoxazole and trimethoprim. It was positive by PCR for strA, strB, int2, sul1 and sul2 genes.

The isolates were examined for quinolone resistance conferring mutations in the topoisomerase genes (gyrA, gyrB, parC and parE), by PCR and sequencing, but no mutations were found. Plasmid-mediated quinolone resistance was screened for by PCR for qnrA and qnrS using primers based on published sequences and for qnrB using primers previously described as FQ1 and FQ2.²

As positive control strains, we used E. coli J53 pMG252, positive for qnrA; E. coli J53 pMG298, positive for qnrB (both strains kindly provided by Dr George Jacoby) and for qnrS E. coli MT102 pbc-H2.6, obtained by electroporating the plasmid pH2.1 (obtained from Dr M. Hata, through the Rikken DNA Bank) into E. coli MT102.¹ H88 tested positive for qnrS and H93 for qnrA. The identification of the gene was confirmed by sequencing of the fragments obtained.

Conjugative transfer was not successful for both strains. Transformation was performed with plasmid DNA from both strains by electroporation into TG1 competent cells (Stratagene). In the case of strain H93, no transformants were obtained.

Hybridization assays on Southern blots showed hybridization of a qnrS probe to a plasmid in strain H88, namely, to an ~20 kb EcoRI fragment or an ~10 kb SmaI fragment of the donor strain and its transformants. In the case of strain H93, hybridization with the qnrA probe was observed to the chromosomal DNA. Although qnrA is generally referred to as plasmid-mediated, this gene is normally found on class I integrons that might jump to the chromosome, which is a possible explanation for the findings in this strain.

This is the first description of plasmid-mediated fluoroquinolone resistance in Scandinavia and in both isolates, the quinolone resistance was found in ESBL-producing multiresistant strains.

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Transparency declarations

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