*J Antimicrob Chemother* 2018; **73**: 1688–1691
doi:10.1093/jac/dky040 Advance Access publication 27 February 2018

Journal of Antimicrobial Chemotherapy

# High admission prevalence of fluoroquinolone resistance in third-generation cephalosporin-resistant Enterobacteriaceae in German university hospitals

**Anna M. Rohde [1,2]*, Miriam Wiese-Posselt[1,2], Janine Zweigner[1–3], Frank Schwab[1,2], Alexander Mischnik[1,4], Harald Seifert[1,3], Petra Gastmeier[1,2] and Winfried V. Kern[1,4] on behalf of the DZIF-ATHOS Study Group†**

*[1]German Center for Infection Research (DZIF), Braunschweig, Germany; [2]Charité – Universitätsmedizin Berlin corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Institute of Hygiene and Environmental Medicine, Berlin, Germany; [3]Institute of Medical Microbiology, Immunology and Hygiene, University Hospital Cologne, Cologne, Germany; [4]Division of Infectious Diseases, Department of Medicine, Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany*

*Corresponding author. Tel: +49-30-450-577603; Fax: +49-30-450-577920; E-mail: anna.rohde@charite.de orcid.org/0000-0002-2190-8267
†Members are listed in the Acknowledgements section.

*Received 25 October 2017; returned 15 January 2018; revised 18 January 2018; accepted 19 January 2018*

**Objectives:** Fluoroquinolone resistance (FQR) in third-generation cephalosporin-resistant Enterobacteriaceae (3GCRE) presents serious limitations to antibiotic therapy. The aim of this study was to investigate whether the FQR proportion among 3GCRE differs between community-acquired (CA) and hospital-acquired (HA) isolates.

**Methods:** In a prospective observational study covering 2014 and 2015, we monitored the occurrence of 3GCRE in adult hospitalized patients in six German university hospitals. 3GCRE clinical isolates were subdivided into CA and HA. Multivariable analysis identified factors associated with *in vitro* non-susceptibility to ciprofloxacin.

**Results:** The dataset included 5721 3GCRE isolates of which 52.9% were HA and 52.7% exhibited FQR. Interestingly, the FQR proportion was higher in CA 3GCRE than in HA 3GCRE (overall, 60.1% versus 46.2%, respectively, $P < 0.001$). Multivariable analysis adjusting for age confirmed community acquisition as a risk factor for FQR [adjusted rate ratio (aRR) 1.33, 95% CI 1.17–1.53]. *Escherichia coli* and *Klebsiella* spp. were associated with a much higher FQR proportion than other Enterobacteriaceae species (aRR 8.14, 95% CI 6.86–9.65 and aRR 7.62 with 95% CI 6.74–8.61, respectively).

**Conclusions:** The high FQR proportion observed among CA 3GCRE, particularly in *E. coli* and *Klebsiella* spp., indicates that selection pressure in the outpatient setting needs to be addressed with antibiotic stewardship and other interventions in order to limit further spread of MDR.

## Introduction

Due to delays in effective therapy, infections with MDR bacteria lead to longer hospital stays and are associated with worsened patient outcomes. Thus, antibiotic resistance is not only a growing healthcare problem, it is also a burden on the economic system.[1,2] Of great concern is the emergence of ESBL-producing Enterobacteriaceae because ESBL mediates resistance to penicillin and cephalosporins including ceftriaxone and most other third-generation cephalosporins (3GC).[3] These 3GC-resistant (3GCR) Enterobacteriaceae (3GCRE) are often also resistant to fluoroquinolones (and aminoglycosides). This combined resistance phenotype represents a major limitation in therapeutic options, making carbapenems the drugs of choice in many cases. In Europe, 4.3% of the *Escherichia coli* isolates detected in blood or cerebrospinal fluid showed this MDR pattern.[4] In Germany the proportion appears to be higher: 5.4% of the *E. coli* and 7.1% of the *Klebsiella pneumoniae* isolates from ambulatory care and 8.9% of the *E. coli* and 10.2% of the *K. pneumoniae* isolates from the hospital care sector exhibited this pattern.[5]

The goal of the present study was to investigate potential differences in fluoroquinolone resistance (FQR) between hospital-acquired (HA) and community-acquired (CA) 3GCRE.

## Methods

### Study design and data sources

This study is a secondary analysis of a prospective observational study in the ATHOS (Antibiotic Therapy Optimization Study) project. Hospitalized patients were monitored for the first 3GCRE occurrence in six German university hospitals in 2014 and 2015. Adult patients admitted to acute care services other than psychiatry, dermatology, otorhinolaryngology, ophthalmology, paediatrics and gynaecology were included.

### Microbiological analysis and definitions

Standard laboratory procedures were used for species identification [MALDI-TOF MS or Vitek 2 GN ID card (bioMérieux)] and *in vitro* susceptibility testing [Vitek 2 (bioMérieux)]. Piperacillin, cefotaxime, ceftazidime, ciprofloxacin, imipenem and meropenem MICs were interpreted according to EUCAST breakpoints.[6] Isolates with non-susceptibility to ciprofloxacin were counted as FQR. Isolates detected in specimens taken on days 1–3 (admission day = day 1) were considered to be CA, whereas those detected from day 4 onwards were considered to be HA.[7] Isolates detected in specimens that suggest an impact on patient health, like wound swabs, blood, urine etc., were considered clinically relevant. Infections were defined as detection in clinically relevant specimens with signs of clinical infection and therapeutic intervention.

### Statistical analysis

FQR distribution was tested with the $\chi^2$ test. The FQR proportion was calculated as the number of Enterobacteriaceae with 3GCR and FQR divided by all 3GCRE and was stratified for covariates. The FQR adjusted rate ratios (aRRs) with 95% CIs were calculated with generalized linear logistic regression with a general estimate equation model. The covariates were remodelled as binary variables. The hospital variable was included in all models to account for cluster effects. Only variables with $P < 0.2$ in the Type 3 test in univariable analysis were included in the multivariable model. Stepwise backward variable selection based on the Type 3 test was then used. In a second multivariable model, stepwise forward variable selection based on the Quasilikelihood under the Independence model Criterion (QIC) was employed. For epidemiological reasons, age and gender were included in both multivariable models. $P$ values $<0.05$ were considered significant. The statistical analysis was performed with SAS 9.4 (SAS Institute, Cary, NC, USA).

### Ethics and data protection

Surveillance was performed in accordance with the German Infection Protection Act.[8] The data were entered into an online accessible database

**Table 1.** Descriptive statistics of 5721 3GCRE observations, divided into '3GCR only' Enterobacteriaceae and those with additional FQR ('3GCR + FQR')

| Parameter | Category | 3GCR only, *n* | 3GCR + FQR, *n* | Total, *n* | FQR (%) | *P* |
|---|---|---|---|---|---|---|
| Total | | 2704 | 3017 | 5721 | 52.7 | |
| Age (years) | <46 | 302 | 398 | 700 | 56.9 | 0.032 |
| | 46–55 | 329 | 359 | 688 | 52.2 | |
| | 56–65 | 537 | 582 | 1119 | 52.0 | |
| | 66–75 | 706 | 838 | 1544 | 54.3 | |
| | >75 | 830 | 840 | 1670 | 50.3 | |
| Gender | male | 1479 | 1666 | 3145 | 53.0 | 0.691 |
| | female | 1225 | 1351 | 2576 | 52.5 | |
| Hospital | centre 1 | 383 | 917 | 1300 | 70.5 | <0.001 |
| | centre 2 | 191 | 195 | 386 | 50.5 | |
| | centre 3 | 334 | 553 | 887 | 62.3 | |
| | centre 4 | 702 | 651 | 1353 | 48.1 | |
| | centre 5 | 830 | 413 | 1243 | 33.2 | |
| | centre 6 | 264 | 288 | 552 | 52.2 | |
| Species | *E. coli* | 726 | 1897 | 2623 | 72.3 | <0.001 |
| | *Klebsiella* spp. | 253 | 653 | 906 | 72.1 | |
| | *Enterobacter* spp. | 1006 | 274 | 1280 | 21.4 | |
| | *Citrobacter* spp. | 331 | 84 | 415 | 20.2 | |
| | other Enterobacteriaceae[a] | 388 | 109 | 497 | 21.9 | |
| Specimen | urine | 947 | 1343 | 2290 | 58.7 | <0.001 |
| | TBS | 519 | 265 | 784 | 33.8 | |
| | wound swab | 379 | 312 | 691 | 45.2 | |
| | blood culture | 149 | 233 | 382 | 61.0 | |
| | stool | 55 | 43 | 98 | 43.9 | |
| | other specimens[b] | 655 | 821 | 1476 | 55.6 | |
| Infection status | colonization | 1143 | 1227 | 2370 | 51.8 | 0.220 |
| | infection | 1561 | 1790 | 3351 | 53.4 | |
| Acquisition | CA | 1076 | 1619 | 2695 | 60.1 | <0.001 |
| | HA | 1628 | 1398 | 3026 | 46.2 | |
| Ward category | general ward | 1670 | 2074 | 3744 | 55.4 | <0.001 |
| | ICU | 934 | 782 | 1716 | 45.6 | |
| | intermediate care | 100 | 161 | 261 | 61.7 | |
| Service type | non-surgical | 1438 | 1701 | 3139 | 54.2 | 0.036 |
| | surgical | 1063 | 1121 | 2184 | 51.3 | |
| | haematology/oncology | 203 | 195 | 398 | 49.0 | |

[a]'Other Enterobacteriaceae' includes *Proteus*, *Serratia*, *Hafnia*, *Morganella*, *Providencia*, *Raoultella*, *Pantoea* and *Cedecea* species.
[b]'Other specimens' includes cerebrospinal fluid, punctate, sputum, 'unknown' and 'other'. TBS, tracheobronchial secretion.

whose data protection concept was approved by the data protection commissioner.

## Results

The study included 578 420 admissions over the 2-year period and yielded 5721 clinically relevant 3GCRE isolates. Of these, 3145 (55.0%) were isolated from male patients, 2623 (45.8%) were *E. coli*, 906 (15.8%) were *Klebsiella* spp. and approximately half of all isolates were HA 3GCRE (3026; 52.9%). The overall FQR proportion among 3GCRE was 52.7% ($n = 3017$) (Table 1), ranging between 33.2% and 70.5% from the participating hospitals. CA 3GCRE had a significantly higher proportion of FQR than HA 3GCRE (60.1% versus 46.2%, respectively).

Stepwise backward and stepwise forward variable selection yielded the same multivariable model that included age, gender, *E. coli*, *Klebsiella* spp. and community acquisition as independent factors for FQR detection in 3GCRE (Table 2). CA 3GCRE showed a higher risk of FQR than HA 3GCRE (CA: aRR 1.33, 95% CI 1.17–1.53). *E. coli* and *Klebsiella* spp. showed a very high and significant risk for FQR (aRR 8.14 and 7.62, respectively) compared with other Enterobacteriaceae species. Being female was found to be protective (aRR 0.77, 95% CI 0.72–0.83) and age was not associated with FQR. The infection status (infection versus colonization), ward category and service type were not associated with FQR in the multivariable model.

## Discussion

The somewhat surprising higher FQR proportion in CA 3GCRE was observed among *E. coli*, *Klebsiella* spp. and other Enterobacteriaceae. We think the reasons for these findings are manifold. The data essentially indicate a substantial selection pressure from fluoroquinolones outside the hospital coming from antibiotic consumption in patients and probably in their environment and in food production.

**Table 2.** Results of multivariable logistic regression for the outcome 3GCR + FQR

| Parameter | Category | FQR aRR | 95% CI | *P* |
|---|---|---|---|---|
| Intercept | | 0.29 | 0.22–0.38 | <0.001 |
| Age (years) | <46 | 1.09 | 0.83–1.42 | 0.529 |
| | 46–55 | 1.03 | 0.79–1.33 | 0.849 |
| | 56–65 | 1.00 | 0.85–1.19 | 0.971 |
| | 66–75 | 1.12 | 0.95–1.32 | 0.185 |
| | >75 | 1 = ref. | | |
| Gender | female | 0.77 | 0.72–0.83 | <0.001 |
| | male | 1 = ref. | | |
| *E. coli* | 1: yes | 8.14 | 6.86–9.65 | <0.001 |
| | 0: no | 1 = ref. | | |
| *Klebsiella* spp. | 1: yes | 7.62 | 6.74–8.61 | <0.001 |
| | 0: no | 1 = ref. | | |
| Acquisition | CA | 1.33 | 1.17–1.53 | <0.001 |
| | HA | 1 =ref. | | |

ref., reference.

Patients with isolation of a 3GCRE strain on admission or within two days after the admission day likely represent a group with relevant and perhaps repeated exposures to antibiotics because of infection risks associated with underlying diseases. We excluded MDR screening specimens but clinically relevant cultures may have been ordered intensely in this period. Outpatient management of these patients may have often included oral antibiotics with enhanced coverage for Gram-negative bacteria, and in many cases these drugs will be broad-spectrum cephalosporins or fluoroquinolones rather than amoxicillin or trimethoprim/sulfamethoxazole. Fluoroquinolone use in the month prior to hospital admission was found to be associated with detection of FQR *E. coli* in the first three days of hospital stay.[9] Unfortunately, our dataset does not include patient-based antibiotic consumption data. Interestingly, surveillance data on outpatient antibiotic consumption show enhanced use of oral cephalosporins in Germany compared with other European countries. Fluoroquinolone consumption is comparable with the population-weighted EU mean.[10] However, more fluoroquinolones are prescribed in ambulatory compared with hospital care (total of 21.2 versus 9.3 million DDDs, respectively).[11–13] A similar relationship was found in England.[14] Excessive fluoroquinolone use in ambulatory care may explain the higher FQR proportion observed in CA 3GCRE. *E. coli* and *Klebsiella* spp. were found to have an increased risk of being FQR. In infections with both species, in addition to fluoroquinolone use, aminoglycoside use was shown to be an independent risk factor for FQR occurrence in 3GCRE.[15] Such co-selection processes would not be expected to be relevant in newly admitted patients and are less likely to explain the high FQR proportion in our dataset.

This study is the first to compare FQR in CA and HA 3GCRE. Limitations may be the definition of community acquisition including two days after the admission day, which may have been too broad, and ignoring previous admissions, outpatient treatments or travel history. Also, we did not include cephalosporin-susceptible Enterobacteriaceae and therefore cannot distinguish whether the increased FQR proportion among CA 3GCRE is specific for 3GCRE or a general phenomenon. Moreover, the database included only initial detections of 3GCRE. Missing follow-up data may lead to an underestimation of HA 3GCRE with and without FQR. In addition, frequent carbapenem use in hospital patients for suspected MDR pathogens may suppress or even eliminate colonizing MDR Gram-negative bacteria which may result in fewer detections of FQR 3GCRE. As the microbiological data of the isolates were generated by hospital routine diagnostics, 3GCR mechanism data are not available. From a parallel admission prevalence study we know that the majority of 3GCR in the endogenous bacteria of our patients was caused by ESBL (67% CTX-M1 group and 17% CTX-M9 group) and about 10% was caused by AmpC genotypes.[16]

We still consider the finding that community acquisition rather than hospital acquisition of 3GCRE is associated with FQR significant and important. It confirms the need for more data from and antibiotic stewardship activities in the outpatient setting. General practitioner-based surveillance modules informing them about their antibiotic prescription behaviour and dedicated antibiotic stewardship (ABS) programmes may increase adherence to prescription guidelines.[17] Voluntary (group) education for practitioners has been shown to reduce inappropriate antibiotic prescriptions.[18] In addition, non-prescribed use of antibiotics is of concern. In representative surveys, about 5%–8% of the German

participants reported antibiotic usage that had not been prescribed (EU mean, 7%).[19,20] Educating the population on the development of antibiotic resistance, restricting use to prescription antibiotics worldwide and removing antibiotics from online pharmacy portfolios may sustain their therapeutic effectiveness.

## Acknowledgements

We would like to acknowledge all members of the DZIF-ATHOS Study Group.

### *Members of the DZIF-ATHOS Study Group*

Sabina Armean, Tübingen; Michael Behnke, Berlin; Dirk Busch, Munich; Susanne Feihl, Munich; Gesche Först, Freiburg; Federico Foschi, Tübingen; Meyke Gillis, Cologne; Axel Hamprecht, Cologne; Dorothea Hansen, Cologne; Georg Häcker, Freiburg; Markus Heim, Munich; Martin Hug, Freiburg; Klaus Kaier, Freiburg; Johannes Knobloch, Lübeck; Axel Kola, Berlin; M. Fabian Küpper, Freiburg; Georg Langebartels, Cologne; Andrea Liekweg, Cologne; Hans-Peter Lipp, Tübingen; Mathias Nordmann, Berlin; Birgit Obermann, Lübeck; Luis-Alberto Peña-Diaz, Berlin; Silke Peter, Tübingen; Christiane Querbach, Munich; Jan Rupp, Lübeck; Christian Schneider, Tübingen; Christin Schröder, Berlin; Wiebke Schröder, Tübingen; Katrin Spohn, Tübingen; Michaela Steib-Bauert, Freiburg; Evelina Tacconelli, Tübingen; Jörg J. Vehreschild, Cologne; Ulrich vor dem Esche, Freiburg; Mathias Willmann, Tübingen.

## Funding

This work was supported by the German Center for Infection Research (grant number TTU 08.801). Microbiology data (species identification and *in vitro* susceptibility testing) were generated as part of routine diagnostics.

## Transparency declarations

None to declare.

## References

**1** Leistner R, Gurntke S, Sakellariou C *et al*. Bloodstream infection due to extended-spectrum β-lactamase (ESBL)-positive *K. pneumoniae* and *E. coli*: an analysis of the disease burden in a large cohort. *Infection* 2014; **42**: 991–7.

**2** Hwang AY, Gums JG. The emergence and evolution of antimicrobial resistance: impact on a global scale. *Bioorg Med Chem* 2016; **24**: 6440–5.

**3** WHO. *Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics*. http://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/.

**4** ECDC. *Antimicrobial Resistance Surveillance in Europe 2015. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net)*. Stockholm, Sweden: ECDC, 2017.

**5** Robert Koch Institut. *Antibiotic Resistance Surveillance in Primary Care*. https://ars.rki.de/Docs/Multiresistance/KRINKO/KRINKO_PR.pdf.

**6** EUCAST. *Breakpoint Tables for Interpretation of MICs and Zone Diameters, Version 4.0*. http://www.eucast.org.

**7** CDC. *Multidrug-Resistant Organism & Clostridium difficile Infection (MDRO/CDI) Module*. https://www.cdc.gov/nhsn/pdfs/pscmanual/12pscmdro_cdadcurrent.pdf.

**8** Federal Ministry of Justice and Consumer Protection. *German Infection Protection Act, §23*. 2001.

**9** Richard P, Delangle MH, Raffi F *et al*. Impact of fluoroquinolone administration on the emergence of fluoroquinolone-resistant Gram-negative bacilli from gastrointestinal flora. *Clin Infect Dis* 2001; **32**: 162–6.

**10** ECDC. *Surveillance of Antimicrobial Consumption in Europe 2012*. https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/antimicrobial-consumption-europe-esac-net-2012.pdf.

**11** Bätzing-Feigenbaum J, Schulz M, Schulz M *et al*. *Entwicklung des Antibiotikaverbrauchs in der ambulanten vertragsärztlichen Versorgung 2008 - 2014*. Berlin, Germany: Zentralinstitut für die kassenärztliche Versorgung in Deutschland (Zi), 2015.

**12** Federal Office of Statistics. *Einrichtungen, Betten und Patientenbewegung*. DESTATIS, 2017.

**13** Schweickert B, Feig M, Behnke M *et al*. Antibiotic consumption in German acute care hospitals: first data of a new web-based national surveillance system. In: *Abstracts of the Twenty-seventh European Congress of Clinical Microbiology and Infectious Diseases, Vienna, Austria, 2017*. Abstract EV0425. ESCMID, Basel, Switzerland.

**14** Dingle KE, Didelot X, Quan TP *et al*. Effects of control interventions on *Clostridium difficile* infection in England: an observational study. *Lancet Infect Dis* 2017; **17**: 411–21.

**15** Lautenbach E, Strom BL, Bilker WB *et al*. Epidemiological investigation of fluoroquinolone resistance in infections due to extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*. *Clin Infect Dis* 2001; **33**: 1288–94.

**16** Hamprecht A, Rohde AM, Behnke M *et al*. Colonization with third-generation cephalosporin-resistant Enterobacteriaceae on hospital admission: prevalence and risk factors. *J Antimicrob Chemother* 2016; **71**: 2957–63.

**17** Batzing-Feigenbaum J, Schulz M, Schulz M *et al*. Outpatient antibiotic prescription. *Dtsch Arztebl Int* 2016; **113**: 454–9.

**18** Welschen I, Kuyvenhoven MM, Hoes AW *et al*. Effectiveness of a multiple intervention to reduce antibiotic prescribing for respiratory tract symptoms in primary care: randomised controlled trial. *BMJ* 2004; **329**: 431.

**19** Paget J, Lescure D, Versporten A *et al*. *Antimicrobial Resistance and Causes of Non-Prudent Use of Antibiotics in Human Medicine in the EU*. Luxembourg: Publications Office of the European Union, 2017.

**20** Schneider S, Salm F, Schroder C *et al*. [Antibiotic intake and resistance development—knowledge, experience and behavior among the German general population]. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* 2016; **59**: 1162–70.