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Comparative dynamics of the emergence of fluoroquinolone resistance in staphylococci from the nasal microbiota of patients treated with fluoroquinolones according to their environment☆

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ABSTRACT

Fluoroquinolone-resistant staphylococci (FQRS) are primarily selected in the nasal microbiota during fluoroquinolone (FQ) treatment. To gain insight into the dynamics of the emergence of FQRS, 49 hospitalised patients (HPs) and 62 community patients (CPs) treated with FQs were studied. Nasal swabs were collected before (T0), at the end of (T1) and 1 month after (T2) FQ treatment. FQRS were identified by mass spectrometry. Antibiotic resistance was determined. Pre- and post-exposure staphylococci populations were compared phenotypically and by MLST to determine the origin of FQRS. At T0, 33/49 HPs (67%) and 24/62 CPs (39%) carried FQRS (OR = 3.3, 95% CI: 1.4–7.9; $P < 0.001$). Among patients with no FQRS at T0, 15/16 HPs (94%) and 16/38 CPs (42%) had FQRS detected at T1 and/or T2 (OR = 19.6, 95% CI: 2.5–902; $P < 0.001$). Among FQRS having emerged, co-resistance to meticillin was detected in 87% and 82% of HPs and CPs, respectively. No selection of resistance emerging from the initial microbiota was evidenced. FQRS showed decreased species diversity in favour of *Staphylococcus haemolyticus* and *Staphylococcus epidermidis*. As a consequence of FQ treatment, acquisition of FQRS in the nasal microbiota is frequent in the community and almost inevitable in hospitals. Acquisition from extranasal sites prevails. A restriction in species diversity in favour of more pathogenic and resistant species occurs. This highlights the major impact of FQ treatment on nasal microbiota, the role of the ecological environment in the emergence of FQRS, and the high-risk of dissemination of resistant staphylococci.

1. Introduction

Staphylococci resistance to antibiotics is a growing therapeutic problem both in the community and in hospitals, involving all antibiotics including fluoroquinolones (FQs) [1,2]. FQs are widely used worldwide for the treatment of community- and hospital-acquired infections because of their large spectrum, excellent tissular diffusion and low toxicity profile. The global increase in the use of FQs has led to the rapid emergence of FQ resistance, responsible for therapeutic failures, increased hospitalisations and costs, and the increase in the use of alternative antibiotics [3,4]. In addition, FQ use is associated with the emergence of multiresistant organisms, including meticillin-resistant *Staphylococcus aureus* (MRSA) [5]. Resistance to FQs may be the result of direct selection at the site of infection or in the microbiota [6]. However, the role of the microbiota as a major source of bacterial resistance may be more important than the infectious foci as higher numbers of bacteria as well as various species are present, allowing multiple horizontal gene transfers to pathogens as well as direct infection by commensal bacteria [7]. *S. aureus* and coagulase-negative staphylococci (CoNS) are major components of the human skin and mucosal flora, including the nasal microbiota [8]. The anterior nares are

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indeed the primary colonisation site of *S. aureus* [9] and are also a major reservoir for CoNS [8]. Both *S. aureus* and CoNS are also major pathogens in their own right [10,11], and *S. aureus* nasal carriage has been shown to precede infection [12]. Because commensals are repeatedly impacted by antibiotics and may directly cause infection, studying the emergence of resistance in commensal staphylococci is crucial.

Epidemiological studies have found that up to one-third of patients carry FQ-resistant staphylococci (FQRS) upon admission to French hospitals [13,14]. After a few days of FQ treatment, most hospitalised patients carry FQRS in their nares, with potential severe clinical complications in case of infection and a high risk of dissemination [14–17]. However, the impact of FQ treatment on the emergence of resistance in patients treated in the community has never been studied. Little is known either about the mechanisms involved in the emergence of resistance during FQ treatment or the dynamics of acquisition of FQRS (through exogenous acquisition, de novo mutation or the selection of a minority resistant mutant) or the impact of FQs on the diversity of the nasal staphylococci population.

In this study, a unique collection of nasal swabs from patients having received FQ treatment was used to compare the dynamics of the emergence of resistance in nasal staphylococci from community patients (CPs) and hospitalised patients (HPs) and to determine the most likely origin of FQRS having emerged after treatment in both patient groups. The impact of FQ treatment on the diversity of the staphylococci populations colonising the nasal microbiota in both groups was also compared.

2. Materials and methods

2.1. Subjects and study design

Samples collected in two large clinical trials currently closed for inclusion and whose results are or will be published separately were used [14]. The first trial, StaphMRG (http://clinicaltrials.gov/ ; ClinicalTrials.gov identifier: NCT01489878), was a prospective, observational, open study performed with 34 university-affiliated general practitioners from the Paris region of France that aimed to compare the acquisition rates of meticillin-resistant CoNS (MR-CoNS) nasal carriage in CPs treated with either a β-lactam, a macrolide, Synergistin or a FQ. In the current work, samples collected only from patients receiving a FQ were used. Patients were adults (≥18 years), did not have a history of hospitalisation within the previous 6 months and had not received antibiotics in the previous 2 months.

The second study, FQEMERG (ClinicalTrials.gov identifier: NCT01209247), was a prospective, two-centre study that aimed to determine the incidence and risk factors involved in the emergence of FQ resistance in the microbiota of HPs treated with a FQ. Patients included in the FQEMERG study were hospitalised adults (≥18 years) receiving FQ treatment for at least 1 day; additional information and epidemiological data can be found elsewhere [14]. Both studies were approved by the French National Ethics Committee and patients had given informed consent. In the interest of this specific work, only nasal samples from FQ-treated patients in the StaphMRG and FQEMERG studies were used. Both studies had similar designs: nasal swabs had been collected before (T0), within 5 days after the end of (T1) and ca. 30 days (21–45 days) after the end of FQ treatment (T2). Patients whose samples did not have ≥5 CFU when swabs were spread on Chapman agar containing no antibiotics were excluded. Altogether, 62 FQ-treated CPs and 49 FQ-treated HPs were included from the StaphMRG and FQEMERG studies, respectively.

2.2. Isolation of fluoroquinolone-resistant staphylococci from nasal swab samples

All nasal swabs had been discharged immediately in 1 mL of brain–heart infusion broth with 10% glycerol upon reception and stored at −80 °C until analysis. The methodology used for sampling, isolation and characterisation of FQRS were strictly the same for the CPs and HPs [14]. To detect FQRS, 100 μL of the initial frozen sample was plated on two Chapman agar plates (bioMérieux, Marcy-l'Étoile, France) with and without 1 mg/L ciprofloxacin and was incubated for 48 h at 37 °C. The number of CFU/plate was determined. Based on this first phenotypic screening, it was determined whether each subject carried ciprofloxacin-susceptible and/or -resistant staphylococci on each sampling day. Subjects who experienced the emergence of resistance, defined as carriage of FQ-susceptible staphylococci (FQSS) at T0 and FQRS at T1 and/or T2 were subsequently studied in detail. For these patients, four distinct colonies were randomly picked on each of the plates (Chapman with and without antibiotics on each of the collection days) for further analysis and were subcultured on trypticase soy agar.

2.3. Species identification and antibiotic susceptibility patterns

For each of the four colonies, organisms were identified to species level by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (microflex™ MALDI-TOF Mass Spectrometer; Bruker Daltonics, Wissembourg, France) according to the manufacturer's instructions. Mass spectra were analysed using the flexControl software (Bruker Daltonics). Quality indices (QI) were allocated to assess the accuracy of species identification [18]. Only spectra with a QI > 2 were considered reliable for identification. Antibiotic susceptibility patterns to 12 antibiotics (penicillin, oxacillin, kanamycin, tobramycin, gentamicin, erythromycin, lincomycin, tetracycline, fusidic acid, fosfomycin, rifampicin and trimethoprim/sulfamethoxazole) were determined using the disc diffusion method and the results were interpreted using the breakpoints recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [19]. Meticillin resistance was also detected by latamoxef and cefoxitin disc diffusion and was then confirmed by triplex real-time PCR (RT-PCR) including the *mecA* gene. The *femA* gene of *S. aureus* and the signature of staphylococci in the *rrs* gene were also amplified in this triplex RT-PCR [20]. Minimum inhibitory concentrations (MICs) of norfloxacin, ofloxacin, ciprofloxacin and levofloxacin were measured by the agar dilution method and the results were interpreted using the breakpoints established by EUCAST. FQRS were defined by the detection of CoNS or *S. aureus* in the nasal microbiota with a ciprofloxacin MIC of >1 mg/L [19]. Among the four colonies picked, isolates from a given patient on a given day were considered as duplicates, and thus were not analysed further, if they had identical MS species identifications and identical antibiotic susceptibility patterns.

2.4. DNA extraction, amplification and sequencing of the quinolone resistance-determining region (QRDR)

DNA extraction was performed for all FQRS and FQ-susceptible isolates of the different reference species using a MagNA Pure LC DNA Isolation Kit (Roche, Mannheim, Germany) following the manufacturer's instructions. Internal fragments corresponding to the QRDR region of the *gyrA*, *grlA* and *grlB* genes were amplified in an I-cycler (Bio-Rad, Marnes-la-Coquette, France) using primers designed in-house and listed in Supplementary Table S1. *gyrB* mutations have not been described to be responsible for FQ resistance in staphylococci, therefore this gene was not amplified. The amplification mixture contained 100 ng of DNA, the two