Association between quinolone use in food animals and gonococcal resistance to ciprofloxacin: an ecological study

Natalia Gonzalez

Said Abdellati

Sheeba Manoharan-Basil

Chris Kenyon[1,2*]

[1]HIV/STI Unit, Institute of Tropical Medicine, Antwerp, Belgium; [2]Division of Infectious Diseases and HIV Medicine, University of Cape Town, Anzio Road, Observatory 7700, South Africa

*Corresponding author. HIV/STI Unit, Institute of Tropical Medicine, Antwerp, 2000, Belgium. Tel: +32 3 2480796; Fax: +32 3 2480831; E-mail: ckenyon@itg.be

Running title: Quinolone use in food animals and gonococcal resistance

Word count: Text: 1659 words, Abstract: 27

Abstract
We found evidence of a positive country-level association between the intensity of quinolone use for animal husbandry and the prevalence of ciprofloxacin resistance in *Neisseria gonorrhoeae*.

Keywords: *N. gonorrhoeae*, antimicrobial consumption, AMR, resistance, quinolone, one-health

Introduction

Why has gonococcal resistance to fluoroquinolones emerged so explosively in East Asia? The prevalence of ciprofloxacin resistance in China, for example, increased from 10% to 95% between 1996 and 2003 [1]. In comparison, the country-level median prevalence of ciprofloxacin resistance in 2009 was 24% in the Americas and 6% in Africa [2]. Whilst differences in the intensity of exposure to fluoroquinolones likely play a key role in explaining these differences in resistance, it is not clear what pathways are most important. Quinolones used to treat *N. gonorrhoeae* can select for antimicrobial resistance (AMR) directly (direct selection). Because *N. gonorrhoeae* is asymptomatic for the majority of the time it circulates in a population, quinolones used for other indications can also select for AMR (bystander selection) [3]. *N. gonorrhoeae* can also acquire resistance conferring genes from commensal *Neisseria* [4]. Quinolone used for any indication could select for ciprofloxacin resistance in commensals and subsequently in *N. gonorrhoeae* via this indirect selection pathway [5, 6]. Studies have provided support for each of these pathways in the genesis of gonococcal AMR [4-6]. In particular, a number of studies have established that country level consumption of quinolones is a predictor of ciprofloxacin resistance [2, 3]. Certain Asian countries such as China, Malaysia and the Philippines have been noted to be outliers in these analyses [2]. In 2009, for example, these 3 countries had close to 100% resistance to ciprofloxacin, but all consumed less than half the volume of quinolones consumed by the United States where less than 10% of gonococcal isolates were resistant to ciprofloxacin [2].

There is also an increasing body of evidence to suggest that quinolone resistance in *N. gonorrhoeae* in Asia and elsewhere is part of a syndemic of resistance affecting a

range of gram negatives including *Escherichia coli* and *Pseudomonas spp*. [7]. Further evidence for the syndemic perspective comes from studies which show that the prevalence of quinolone resistance is considerably higher (up to 16-fold higher) in a number of gram negatives in Asia than in Europe and the Americas [8-11]. Between 1998 and 2009, for example the prevalence of ciprofloxacin resistance in *Shigella* increased from 0% to 29% in Asia compared to 0% to 0.6% in Europe-America [8]. This syndemic of quinolone resistance may include *N. meningitidis* and various species of commensal *Neisseria* [12]. Recent studies from Shanghai, for example, have found the prevalence of ciprofloxacin resistance to be 99% in *N. gonorrhoeae*, 100% in commensal *Neisseria* and 66% in *N. meningitidis* [6, 13, 14]. These findings suggest that quinolone exposure other than that used for human infections, may be playing a role in the genesis and spread of quinolone resistance. One such possibility is quinolones used for animal husbandry.

Quinolone use in animals has been linked to AMR in a number of gram-negative pathogens circulating in humans [15, 16]. This use of quinolones could induce resistance in *N. gonorrhoeae* directly or indirectly. Direct selection on *N. gonorrhoeae* would occur via human ingestion of quinolone residues in meat or water/soil contaminated by animal manure [17]. Quinolones have been found to show very low biodegradability in the environment [17, 18]. Selection could also occur indirectly where quinolones select for resistance in commensal *Neisseria* which is then transformed into *N. gonorrhoeae*. Commensal *Neisseria* have been found in the resident microbiomes of a range of food animals including chickens, cows, sheep and goats [19-22]. The selection of quinolone resistance in commensal *Neisseria* could thus occur in the animals or humans. Of note the indirect pathway

has been shown to be important in the genesis and spread of cephalosporin resistance (mainly via the spread of plasmids) in various gram-negative bacteria such as *E. coli [15]*.

An important finding in this field is that antimicrobial concentrations up to 230-fold lower than the minimal inhibitory concentration (MIC) are capable of inducing AMR in bacteria such as *E. coli* and *Salmonella enterica spp.* [23, 24]. Whilst such experiments have not been conducted in *Neisseria spp*., concentrations of ciprofloxacin as low as 0.1 µg/L have been shown to able to select for resistance in other gram negative bacteria [23, 25]. Quinolone concentrations in meat, water and environmental samples have been found to far exceed this threshold in a number of locales: cultivated fish in China (ofloxacin 967 µg/kg) [26], sediment from dam used for aquaculture and drinking water in China (ciprofloxacin 557 µg/kg) [26], cow's milk from 10 provinces in China (quinolones 23 µg/kg) [27] and chicken meat and eggs in China (quinolones 0.4 µg/kg to 624 µg/kg) [28].

These findings provided the motivation to assess at a country level if the prevalence of gonococcal ciprofloxacin resistance is associated with the concentration of quinolones used in food animal production.

Methods

Quinolone use for animal food production data. We obtained the country level consumption of quinolones for animal food production in the year 2013 from a systematic review on this topic performed by Broeckel et al. [29]. This study calculated the volume of antimicrobials (in tons) by class of antimicrobial in 38

countries in the year 2013. Four categories of animals were included: chicken, cattle, pigs and small ruminants (sheep and goats), which together account for the overwhelming majority of terrestrial animals raised for food [15, 29].

We used this data to calculate the number of milligrams of quinolones used for animal food production/population correction unit (PCU) (a kilogram of animal product) in the year 2013. The data for the tonnage of food animals produced per country and year in the year 2013 was taken from the Food and Agriculture Organization estimates (http://www.fao.org/faostat/en/?#data/).

Quinolone consumption in humans. Data from IQVIA were used as a measure of national antimicrobial drug consumption. IQVIA uses national sample surveys that are performed by pharmaceutical sales distribution channels to estimate antimicrobial consumption from the volume of antibiotics sold in retail and hospital pharmacies. The sales estimates from this sample are projected with use of an algorithm developed by IQVIA to approximate total volumes for sales and consumption. Quinolone consumption (Moxifloxacin, Ciprofloxacin, Gemifloxacin, Ofloxacin, Levofloxacin, Lomefloxacin, Norfloxacin, Enoxacin, Gatifloxacin, Trovafloxacin, Sparfloxacin) estimates are reported as the number of standard doses (a dose is classified as a pill, capsule, or ampoule) per 1000 population per year [2].

***N. gonorrhoeae* ciprofloxacin resistance data.** The percent of isolates per country that were resistant to ciprofloxacin in the year 2014 (the year following the quinolone consumption variables) was taken from the WHO Global Gonococcal Antimicrobial Surveillance Programme (GASP). GASP is a collaborative global network of regional

and subregional reference laboratories that monitors gonococcal AMR in participating countries. The full GASP methodology, including suggested sampling strategy, laboratory techniques, external quality assurance, and internal quality control mechanisms has been published elsewhere [30]. A minimum inhibitory concentration (MIC) breakpoint of ≥1μg/ml was used to define resistance to ciprofloxacin [30]. In the case of 4 countries, data was not available for the year 2014. We used the data for the first subsequent year with available data – 2016 (Czechia, Luxembourg) and 2017 (Finland, Switzerland).

Statistical analysis

Spearman's correlation was used to assess the association between the prevalence of ciprofloxacin resistance in *N. gonorrhoeae* and the two independent variables – quinolone use for animals and quinolone consumption by humans. Linear regression was used to assess the country-level association between the percent of *N. gonorrhoeae* isolates with ciprofloxacin resistance and the two independent variables in 3 models. We started by assessing the association between ciprofloxacin resistance and quinolone consumption in humans (Model-1). We then assessed the association between ciprofloxacin resistance and quinolone use in animals (Model-2). Finally in Model-3 we evaluated the effect of both independent variables on ciprofloxacin resistance. Stata 16.0 was used for all analyses. A p-value of <0.05 was considered statistically significant.

Results

No quinolone consumption data was available for two countries (Iran and Nepal). There was considerable variation in quinolone use for food animals in the 36

countries with data (median 1.9 mg quinolones/PCU (IQR 0.7-6.6 mg/PCU; STable 1). Quinolone exposure in China was higher than all other countries (261.2 mg/PCU).

The prevalence of ciprofloxacin resistance in *N. gonorrhoeae* was positively associated with quinolone use for food animals (ρ=0.47; P=0.004; N=34) but not quinolone consumption in humans (ρ=0.31; P=0.097; N=30).

The model that combined quinolone use in food and humans was a better predictor of gonococcal ciprofloxacin resistance (R^2 =0.30; Model 3) than the models that only included either quinolone use in animals or humans (R^2=0.14 [Model 2] and R^2=0.07 [Model 1], respectively; Table 1).

Discussion

Our ecological analysis found an association between quinolone use for animal husbandry and the prevalence of ciprofloxacin resistance in *N. gonorrhoeae*. Of note the combination of quinolone consumption in humans and food animals provided the best prediction of ciprofloxacin resistance.

Being purely ecological these results are best considered as hypothesis generating findings that require further validation. Further weaknesses of the study include the relatively small number of countries with available data, the lack of longitudinal data on quinolone consumption in animals and the absence of data on quinolone use for aquaculture. The epidemiology of resistance is complex and factors other than the amount of quinolones consumed may influence the level of quinolone resistance.

These include consumption of other classes of antimicrobials, travel by humans and trade of live animals and meat [15].

Further experimentation is required to assess the role of antimicrobials used for different indications in selecting for AMR in *N. gonorrhoeae*. These experiments could assess what the minimum selection concentration of ciprofloxacin is for the selection of ciprofloxacin resistance in *N. gonorrhoeae* and the species of commensal *Neisseria* that inhabit humans and their livestock [12, 23]. It would be prudent to assess how efficiently any ciprofloxacin resistance in commensal *Neisseria* resulting from these experiments, could be transformed into *N. gonorrhoeae* [12]. Modelling studies could use the resulting data to model the spread of quinolone resistance in *Neisseria* spp. under different scenarios of quinolone exposure. Finally, intervention studies could be conducted to assess the effect of reducing quinolone use in animal husbandry on the susceptibility of *Neisseria* spp. and other organisms.

Authors' contributions

CK conceptualized the study. CK was responsible for the acquisition, analysis and interpretation of data. CK and read and approved the final draft.

Competing interests

The authors declare that they have no competing interests.

Funding

Nil

Acknowledgements

Nil

Consent for publication

Not applicable

Table 1. Linear regression models testing the country-level association between quinolone consumption in food animals/humans and the prevalence ciprofloxacin resistance expressed as a percentage [coefficients (95% confidence intervals)]

	Model 1	Model 2	Model 3
Quinolones Humans	0.02 (-0.007-0.043)	-	0.2 (0.001-0.046)*
Quinolones Food animals	-	0.2 (0.02-0.38)*	0.2 (0.07-0.40)**
N	30	34	30
R^2	0.07	0.14	0.30

* P-value <0.05, ** P-value <0.01

References

1. Bash MC, McKnew DL, Tapsall JW. Antibiotic Resistance in Neisseria. Antimicrobial Drug Resistance: Springer; 2009. p. 763-82.
2. Kenyon C, Buyze J, Wi T. Antimicrobial consumption and susceptibility of Neisseria gonorrhoeae: a global ecological analysis. Frontiers in medicine. 2018;5:329.
3. Kenyon C, Laumen J, Van Dijck C, De Baetselier I, Abdelatti S, Manoharan-Basil SS, et al. Gonorrhoea treatment combined with population-level general cephalosporin and quinolone consumption may select for Neisseria gonorrhoeae antimicrobial resistance at the levels of NG-MAST genogroup: an ecological study in Europe. J Glob Antimicrob Resist. 2020. Epub 2020/11/19. doi: 10.1016/j.jgar.2020.10.022. PubMed PMID: 33207228.
4. Spratt BG, Bowler LD, Zhang QY, Zhou J, Smith JM. Role of interspecies transfer of chromosomal genes in the evolution of penicillin resistance in pathogenic and commensal Neisseria species. J Mol Evol. 1992;34(2):115-25. Epub 1992/02/01. doi: 10.1007/BF00182388. PubMed PMID: 1556747.
5. Potts CC, Retchless AC, McNamara LA, Marasini D, Reese N, Swint S, et al. Acquisition of ciprofloxacin resistance among an expanding clade of β-lactamase positive, serogroup Y Neisseria meningitidis in the United States. Clinical Infectious Diseases. 2021.
6. Chen M, Zhang C, Zhang X, Chen M. Meningococcal quinolone resistance originated from several commensal Neisseria species. Antimicrobial agents and chemotherapy. 2019.
7. Kenyon C, Manoharan-Basil SS, Van Dijck C. Gonococcal resistance can be viewed productively as part of a syndemic of antimicrobial resistance: an ecological analysis of 30 European countries. Antimicrob Resist Infect Control. 2020;9:97.
8. Gu B, Cao Y, Pan S, Zhuang L, Yu R, Peng Z, et al. Comparison of the prevalence and changing resistance to nalidixic acid and ciprofloxacin of Shigella between Europe–America and Asia–Africa from 1998 to 2009. Int J Antimicrob Ag. 2012;40(1):9-17.
9. Lu P-L, Liu Y-C, Toh H-S, Lee Y-L, Liu Y-M, Ho C-M, et al. Epidemiology and antimicrobial susceptibility profiles of Gram-negative bacteria causing urinary tract infections in the Asia-Pacific region: 2009–2010 results from the Study for Monitoring Antimicrobial Resistance Trends (SMART). Int J Antimicrob Ag. 2012;40:S37-S43.
10. Song J. Antimicrobial resistance control in Asia. AMR control. 2015:41-5.
11. Yam ELY, Hsu LY, Yap EP, Yeo TW, Lee V, Schlundt J, et al. Antimicrobial Resistance in the Asia Pacific region: a meeting report. Antimicrob Resist Infect Control. 2019;8:202. Epub 2020/01/01. doi: 10.1186/s13756-019-0654-8. PubMed PMID: 31890158; PubMed Central PMCID: PMCPMC6921568.
12. Fiore MA, Raisman JC, Wong NRH, Hudson AO, Wadsworth CB. Exploration of the Neisseria Resistome Reveals Resistance Mechanisms in Commensals That May Be Acquired by N. gonorrhoeae through Horizontal Gene Transfer. Antibiotics-Basel. 2020;9(10). doi: ARTN 656
10.3390/antibiotics9100656. PubMed PMID: WOS:000584161000001.
13. Shen YF, Chen ML. Prevalence, sequence type, and quinolone resistance of Neisseria lactamica carried in children younger than 15 years in Shanghai, China. Journal of Infection. 2020;80(1):61-8. doi: 10.1016/j.jinf.2019.08.020. PubMed PMID: WOS:000507459600008.
14. Yang Y, Liao MM, Gu WM, Bell K, Wu L, Eng NF, et al. Antimicrobial susceptibility and molecular determinants of quinolone resistance in Neisseria gonorrhoeae isolates from Shanghai. J Antimicrob Chemoth. 2006;58(4):868-72. doi: 10.1093/jac/dkl301. PubMed PMID: WOS:000240587400028.

15. Authority EFS. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2018/2019. Efsa J. 2021;19(4).
16. Muloi D, Ward MJ, Pedersen AB, Fevre EM, Woolhouse ME, van Bunnik BA. Are food animals responsible for transfer of antimicrobial-resistant Escherichia coli or their resistance determinants to human populations? A systematic review. Foodborne pathogens and disease. 2018;15(8):467-74.
17. Ben Y, Fu C, Hu M, Liu L, Wong MH, Zheng C. Human health risk assessment of antibiotic resistance associated with antibiotic residues in the environment: A review. Environ Res. 2019;169:483-93. Epub 2018/12/12. doi: 10.1016/j.envres.2018.11.040. PubMed PMID: 30530088.
18. Cantas L, Shah SQ, Cavaco LM, Manaia CM, Walsh F, Popowska M, et al. A brief multi-disciplinary review on antimicrobial resistance in medicine and its linkage to the global environmental microbiota. Front Microbiol. 2013;4:96. Epub 2013/05/16. doi: 10.3389/fmicb.2013.00096. PubMed PMID: 23675371; PubMed Central PMCID: PMCPMC3653125.
19. Răpuntean S, FiŃ N, Chirilă F, Nadăş G, Cosmina C, Călina D, et al. PHYSICAL FACTORS ACTION ON NEISSERIA SPP. STRAINS ISOLATED FROM ANIMALS. Bulletin UASVM. 2008;65:1.
20. Sneath P, Barrett S. A new species of Neisseria from the dental plaque of the domestic cow, Neisseria dentiae sp. nov. Letters in applied microbiology. 1996;23(5):355-8.
21. Kayalvizhi N, Anthony T, Gunasekaran P. Characterization of the Predominant Bacteria Associated with Sheep and Goat Skin. Journal of the American Leather Chemists Association. 2008;103(06):182-7.
22. Liu G, Tang CM, Exley RM. Non-pathogenic Neisseria: members of an abundant, multi-habitat, diverse genus. Microbiology (Reading). 2015;161(7):1297-312. Epub 2015/03/31. doi: 10.1099/mic.0.000086. PubMed PMID: 25814039.
23. Gullberg E, Albrecht LM, Karlsson C, Sandegren L, Andersson DI. Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals. MBio. 2014;5(5).
24. Stanton IC, Murray AK, Zhang L, Snape J, Gaze WH. Evolution of antibiotic resistance at low antibiotic concentrations including selection below the minimal selective concentration. Communications biology. 2020;3(1):1-11.
25. Gullberg E, Cao S, Berg OG, Ilbäck C, Sandegren L, Hughes D, et al. Selection of resistant bacteria at very low antibiotic concentrations. PLoS Pathog. 2011;7(7):e1002158.
26. Huang L, Mo Y, Wu Z, Rad S, Song X, Zeng H, et al. Occurrence, distribution, and health risk assessment of quinolone antibiotics in water, sediment, and fish species of Qingshitan reservoir, South China. Sci Rep. 2020;10(1):15777. Epub 2020/09/27. doi: 10.1038/s41598-020-72324-9. PubMed PMID: 32978412; PubMed Central PMCID: PMCPMC7519050.
27. Zheng N, Wang J, Han R, Xu X, Zhen Y, Qu X, et al. Occurrence of several main antibiotic residues in raw milk in 10 provinces of China. Food Additives & Contaminants: Part B. 2013;6(2):84-9.
28. Yang Y, Qiu W, Li Y, Liu L. Antibiotic residues in poultry food in Fujian Province of China. Food Additives & Contaminants: Part B. 2020;13(3):177-84.
29. Van Boeckel TP, Glennon EE, Chen D, Gilbert M, Robinson TP, Grenfell BT, et al. Reducing antimicrobial use in food animals. Science. 2017;357(6358):1350-2.

30. Wi T, Lahra MM, Ndowa F, Bala M, Dillon JR, Ramon-Pardo P, et al. Antimicrobial resistance in Neisseria gonorrhoeae: Global surveillance and a call for international collaborative action. PLoS Med. 2017;14(7):e1002344. Epub 2017/07/08. doi: 10.1371/journal.pmed.1002344. PubMed PMID: 28686231; PubMed Central PMCID: PMCPMC5501266.