Syndemics of fluoroquinolone resistance in *N. gonorrhoeae, E. coli* and *K. pneumoniae*: a global ecological analysis

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Abstract

Background

It is unclear how important it is to reduce fluoroquinolone consumption in the general population to prevent the spread of fluoroquinolone resistance in *Neisseria gonorrhoeae* (bystander selection).

Methods

We assessed bystander selection by using Spearman's correlation to assess if the country-level prevalence of fluoroquinolone resistance in *N. gonorrhoeae* was correlated with the prevalence of fluoroquinolone resistance in four other gram-negative species - *Acinetobacter baumannii*, Escherichia coli, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Results

Fluoroquinolone resistance in *N. gonorrhoeae* was positively associated with homologous resistance in all 4 species - *A. baumanii*. (ρ=0.61, P=0.0003, *E. coli* (ρ=0.67, P<0.0001), *K. pneumoniae* (ρ=0.52, P=0.0004) and *P. aeruginosa* (ρ=0.40, P=0.0206). Positive associations were also found between the national prevalence of fluoroquinolone resistance and fluoroquinolone consumption in the general population in the preceding year for 4 of the 5 species.

Conclusions

Gonococcal fluoroquinolone resistance can be productively viewed as being part of a syndemic of fluoroquinolone resistance. Strengthening antimicrobial stewardship programs may help retard the spread of fluoroquinolone resistance in *N. gonorrhoeae*.

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Background

Understanding the drivers of antimicrobial resistance (AMR) in *N. gonorrhoeae* is vital to prevent the emergence of untreatable gonorrhoea [1, 2]. Until recently, it was thought that the major determinant of gonococcal AMR was exposure to antibiotics used to treat gonorrhoea [3-5]. Subsequently it was appreciated that *N. gonorrhoeae* is asymptomatic for much or most of the time it circulates in a population. This means that antibiotics used for other indications may cause AMR (bystander selection) [6]. Since gonorrhoea infections tend to cluster with other STIs, this bystander selection may be predominantly due to antibiotics used to treat other STIs (termed the STI bystander theory) [1, 7, 8]. Others have argued that antibiotic consumption for all indications plays a role (community bystander theory; Figure 1) [6, 9-11].

It is vital to establish which of these is the predominant driver of gonococcal AMR. If it is antimicrobials used to treat STIs, then preventing the further emergence of gonococcal AMR could be accomplished by interventions such as antimicrobial stewardship limited to within STI services. If total antibiotic consumption was an important determinant, then stewardship efforts to reduce antibiotic consumption in the whole community would be important [9].

Some studies have found evidence that antimicrobial consumption in the general population is associated with gonococcal AMR [9-11], whereas other have found no association [12]. We recently approached this problem from a novel angle. We tested for a country-level association between the prevalence of fluoroquinolone AMR between *N. gonorrhoeae* and 3 other pathobionts in European countries. We found that the prevalence of fluoroquinolone resistance in *N. gonorrhoeae* was positively

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associated with that in *Pseudomonas aeruginosa, A. baumanii.* and *Klebsiella pneumoniae* as well as being associated with fluoroquinolone consumption in the general population [13]. It was unclear how robust these findings were and if they applied outside the continent of Europe, where the variation in prevalence of fluoroquinolone resistance is much greater [2]. We therefore undertook to repeat the analysis using a global database of countries.

Methods

Data

Antimicrobial Resistance Data

The AMR data for *A. baumanii, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa* was taken from the Center for Disease Dynamics, Economics & Policy's (CDDEP) ResistanceMap database. CDDEP aggregates data on antibiotic resistance from several sources. The data are then harmonized to present similar definitions of resistance across countries and regions to enable comparisons between countries. Further details pertaining to the methodology and definitions used to define antimicrobial resistance can be found at [14]. The list of sources used to obtain the data is provided in STable 1. CDDEP provides data on fluroquinolone resistance for 10 bacterial species but only 4 of these have data for more than 15 countries. We limited our analyses to these 4 species. For each of the species a resistance prevalence estimate from a single year for each country was provided in the dataset. This typically applied to the year 2017 (Table 1).

N. gonorrhoeae: The prevalence of ciprofloxacin resistance per country was obtained 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 [2]. A minimum inhibitory concentration (MIC) breakpoint of $\geq 1\mu g/ml$ was used to define resistance to ciprofloxacin [2]. GASP typically provides data for a number of years for each country. We selected the data applying to the same year as that used to provide the resistance estimate for the comparator species from the CDDEP data.

Fluorouinolone consumption data. 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 [9].

Analyses

For each comparison, Spearman's correlation was used to assess the country-level association between the prevalence of AMR in *N. gonorrhoeae* and each of the other bacterial species in the same year. To test the association between fluoroquinolone

resistance in each species and fluoroquinolone consumption Spearman's correlation was performed using the fluoroquinolone consumption preceding the year the resistance prevalence estimates were obtained from. The statistical analyses were performed in Stata 16.0. A P-value of <0.05 was regarded as significant.

Results

Data was available for between 42 and 60 countries for each of the resistance prevalence estimates per bacterial species (Table 1). For each species considered, the prevalence of fluoroquinolone resistance varied considerably between countries (*N. gonorrhoeae*- median 54.5% [IQR 42.1-76.9]; *E. coli*- median 31.5% [IQR 22.5-47.5]; *K. pneumoniae*- median 44% [IQR 27-62]; *A. baumanii*- median 53.5% [IQR 14-82]; *P. aeruginosa*- median 21.5% [IQR 15-34]; Table 1). Likewise there was significant variation in the consumption of fluoroquinolones- median 613 defined daily doses/1000 inhabitants/year (IQR 375-939).

Syndemics of fluoroquinolone resistance

The prevalence of ciprofloxacin resistance in *N. gonorrhoeae* was positively associated with the prevalence of fluoroquinolone resistance in *A. baumanii.* (ρ=0.61, P=0.0003, N=31, *E. coli* (ρ=0.67, P<0.0001, N=46), *K. pneumoniae* (ρ=0.52, P=0.0004, N=41) and *P. aeruginosa* (ρ=0.40, P=0.0206, N=34; Fig. 1; Table 2).

Likewise, for each species, the prevalence of fluoroquinolone resistance was associated with fluoroquinolone consumption in the preceding year [*A. baumanii.* (ρ=0.68, P=0.0002, N=24), *E. coli* (ρ=0.77, P<0.0001, N=28), *K. pneumoniae* (ρ=0.54, P=0.0046, N=26) and *P. aeruginosa* (ρ=0.62, P=0.0005, N=27); Table 2; Fig. 3]. In

the case of *N. gonorrhoeae* this association was not statistically significant (ρ=0.35, P=0.0620, N=28); Table 2].

Discussion

We found that the prevalence of gonococcal fluoroquinolone resistance was associated with fluoroquinolone resistance in all four of the gram-negative bacteria assessed. Fluoroquinolone AMR was significantly associated with consumption for four of the five bacteria assessed. These findings strengthen the conclusion from our previous study limited to Europe, that found that the fluoroquinolone resistance in *N. gonorrhoeae* can be productively viewed as a syndemic of resistance in other gram negatives [13].

The most parsimonious way to explain these findings is that high fluoroquinolone consumption in the general population is partly responsible for driving fluoroquinolone resistance in all these species. This finding has important consequences. It suggests that reducing fluoroquinolone consumption to below certain thresholds could reduce the probability of resistance sprreading in all of these bacterial species [15, 16]. This may be particularlry important for *N. gonorrhoeae*. As combined high-level resistance to ceftriaxone and azithromycin emerges in *N. gonorrhoeae*, interest is mounting in treating gonorrhoea with single dose oral ciprofloxacin [17, 18]. Studies have confirmed that this strategy results in close to 100% eradication, if preceded by rapid screening for molecular markers of fluoroquinolone resistance [17]. This strategy has also been found to result in reduced use of ceftriaxone thereby reducing selection pressure for cephalosporin resistance [17]. This strategy is, however, not possible in numerous countries in East Asia, where the prevalence of ciprofloxacin resistance is

close to 100% [2]. Studies from Europe have noted declines in ciprofloxacin resistance in the last decade related to changes in treatment protocols and reductions in fluoroquinolone use [13, 19]. These findings provide additional motivation for high fluoroquinolone consumption countries/populations to reduce their fluoroquinolone use in order to preserve these agents as a treatment option for gonorrhoea. Fluoroquinolone use is also rapidly increasing in a number of relatively low consumption countries, such as countries in sub Saharan Africa where the prevalence of gonococcal fluoroquinolone resistance is low [2, 9, 20]. Our findings suggest that these countries may be advised to limit this increase to safer limits.

As in our previous study fluoroquinolone resistance in *N. gonorrhoeae* was most strongly associated with that in *E. coli*. Likewize the association between fluoroquinolone consumption and resistance was strongest in *E. coli*. These findings are congruent with the fact that *E. coli* is the most prevalent of all the bacteria considered in the general human population [6]. This suggests that monitoring fluoroquinolone susceptibility in *E. coli* may function as a useful early warning system to monitor excess fluoroquinolone consumption in populations at risk for AMR [21, 22].

Our analysis is subject to a number of limitations. Chief amongst these are that the AMR prevalence estimates from both GASP and CDDEP are based on various methodologies making cross country comparisons problematic. We did not adjust our analyses for either differences in susceptiblity testing strategies or breakpoints between countries or over time as this information is not provided by GASP or CDDEP. These limitations should however result in a misclassification bias which would

typically result in a bias towards the null hypothesis [23]. This would be expected to reduce the strength of any association found.

The epidemiology of resistance is complex and factors other than the amount of fluroquinolones consumed by humans may influence the level of fluoroquinolone resistance. These include consumption of other classes of antimicrobials, clonal spread of resistance independent of fluoroquinolone consumption, travel by humans and consumption of antimicrobials for acquaculture and livestock production [1, 2, 20, 24, 25]. Future research should strive to develop models that incorporate all these factors so as to be able to better predict and ultimately prevent the further emergence of fluoroquinolone resistance in *N. gonorrhoeae* and other bacteria. In the absence of these studies, the evidence provided by this analysis provides additional evidence to motivate policy makers, prescribers and consumers in high fluoroquinolone consumption populations to reduce consumption.

Authors' contributions

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

Consent for publication

Not applicable

Data availability

The data we used is publicly available from:

https://resistancemap.cddep.org/AntibioticResistance.php

https://apps.who.int/gho/data/node.main.GASPDATA?lang=en

Competing interests

None to declare. All the authors declare that they have no conflicts of interest.

Table 1 Variation in fluoroquinolone consumption (defined daily doses/1000 inhabitants per year) and resistance to fluoroquinolones (%) for 5 bacterial species in 60 countries.

	N	Range	Median	IQR	Year -median (IQR)
Fluoroquinolone consumption[a]	28	136-1486	613	375-939	2016 (2016-2016)
Neisseria gonorrhoeae[b]	46	25-100	54.5	42.1-76.9	2017
Escherichia coli	60	10-84	31.5	22.5-47.5	2017 (2016-2017)
Klebsiella pneumoniae	59	4-87	44	27-62	2017 (2016-2017)
Acinetobacter baumannii	42	0-98	53.5	14-82	2017 (2016-2017)
Pseudomonas aeruginosa	46	5-87	21.5	15-34	2017 (2016-2017)

[a] This data refers to the 28 countries with fluoroquinolone consumption data for the year prior to the year that provided the national resistance estimates for *E. coli* (the bacteria with the greatest N in the CDDEP dataset).
[b] This data is for the 46 countries with data for the prevalence of ciprofloxacin resistance in both *N. gonorrhoeae* and *E. coli* (the bacteria with the greatest N in the CDDEP dataset).

Table 2. Spearman's correlation matrix of prevalence of fluoroquinolone resistance

(%) in 5 bacterial species and fluoroquinolone consumption (defined daily

doses/1000 inhabitants per year) in countries around the world

	Acinetobacter baumannii	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Neisseria gonorrhoeae*	**Fluoroquinolone consumption[a]**
Acinetobacter baumannii	1					
Escherichia coli	0.66*	1				
Pseudomonas aeruginosa	0.76*	0.72*	1			
Klebsiella pneumoniae	0.72*	0.62*	0.91*	1		
Neisseria gonorrhoeae	0.61*	0.67*	0.40*	0.53*	1	
Fluoroquinolone consumption	0.68*	0.77*	0.62*	0.54*	0.35	1

* $P < 0.05$

Figure 1. Conceptual framework for understanding how different forms of bystander selection could result in *N. gonorrhoeae* fluoroquinolone (FQ) resistance. The blue-pathway represents STI bystander selection where the use of fluoroquinolones to treat other STIs (e.g. *Mycoplasma genitalium*) selects for fluoroquinone resistance in *N. gonorrhoeae*. The red-pathway depicts community bystander selection whereby high-levels of consumption of fluoroquinolones for any indication would select for resistance in circulating commensal and pathogenic bacteria. This community bystander selection could also act on *N. gonorrhoeae* and commensal *Neisseria.* Fluoroquinolone resistance could be transferred from commensal Neisseria to N. gonorrhoeae via transformation (stippled arrow).
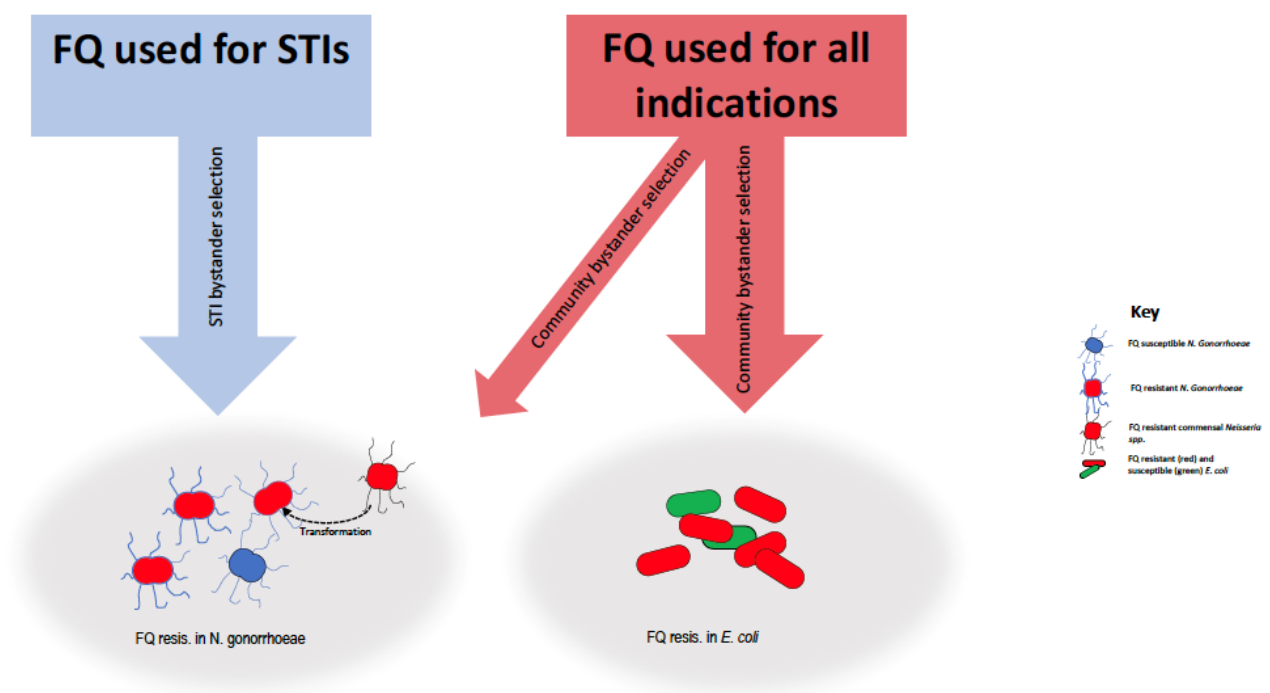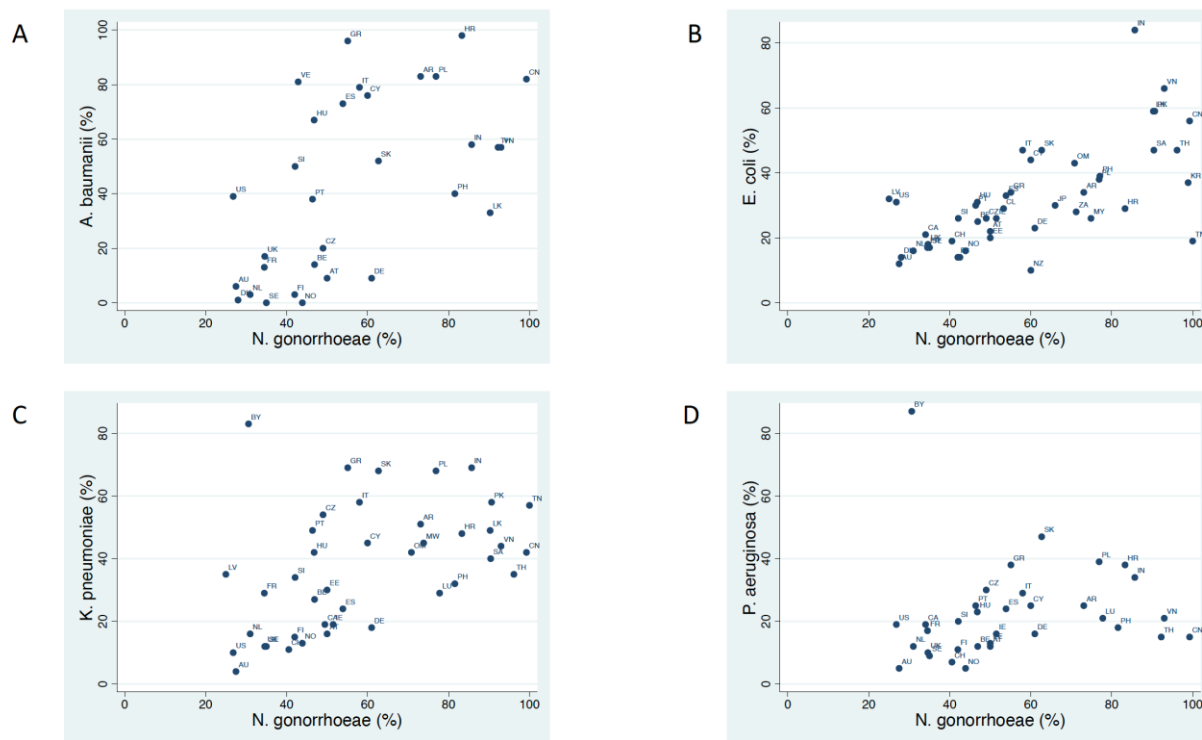
Figure 2. Association between fluoroquinolone resistance in *Neisseria gonorrhoeae* and *Acinetobacter baumanii* (A) *Escherichia coli* (B), *Klebsiella pneumoniae* (C), and *Pseudomonas aeruginosa* (D).

Abbreviations: AT, Austria; BE, Belgium; CN, China; CZ, Czech Republic; DE, Germany; DK, Denmark; EE, Estonia; EL, Greece; ES, Spain; FI, Finland; FR, France; HR, Croatia; HU, Hungary; IE, India; IN, Ireland; IT, Italy; LU, Luxembourg; LV, Latvia; NL, the Netherlands; NO, Norway; PL, Poland; PT, Portugal; SE, Sweden; SI, Slovenia; SK, Slovakia; UK, United Kingdom; US, United States; VN, Venezuela

Figure 3. Association between fluoroquinolone consumption (defined daily doses/1000 inhabitants/year – DID) and resistance in *Acinetobacter baumanii* (A) *Escherichia coli* (B), *Klebsiella pneumoniae* (C), and *Pseudomonas aeruginosa* (D) and *Neisseria gonorrhoeae* (E).
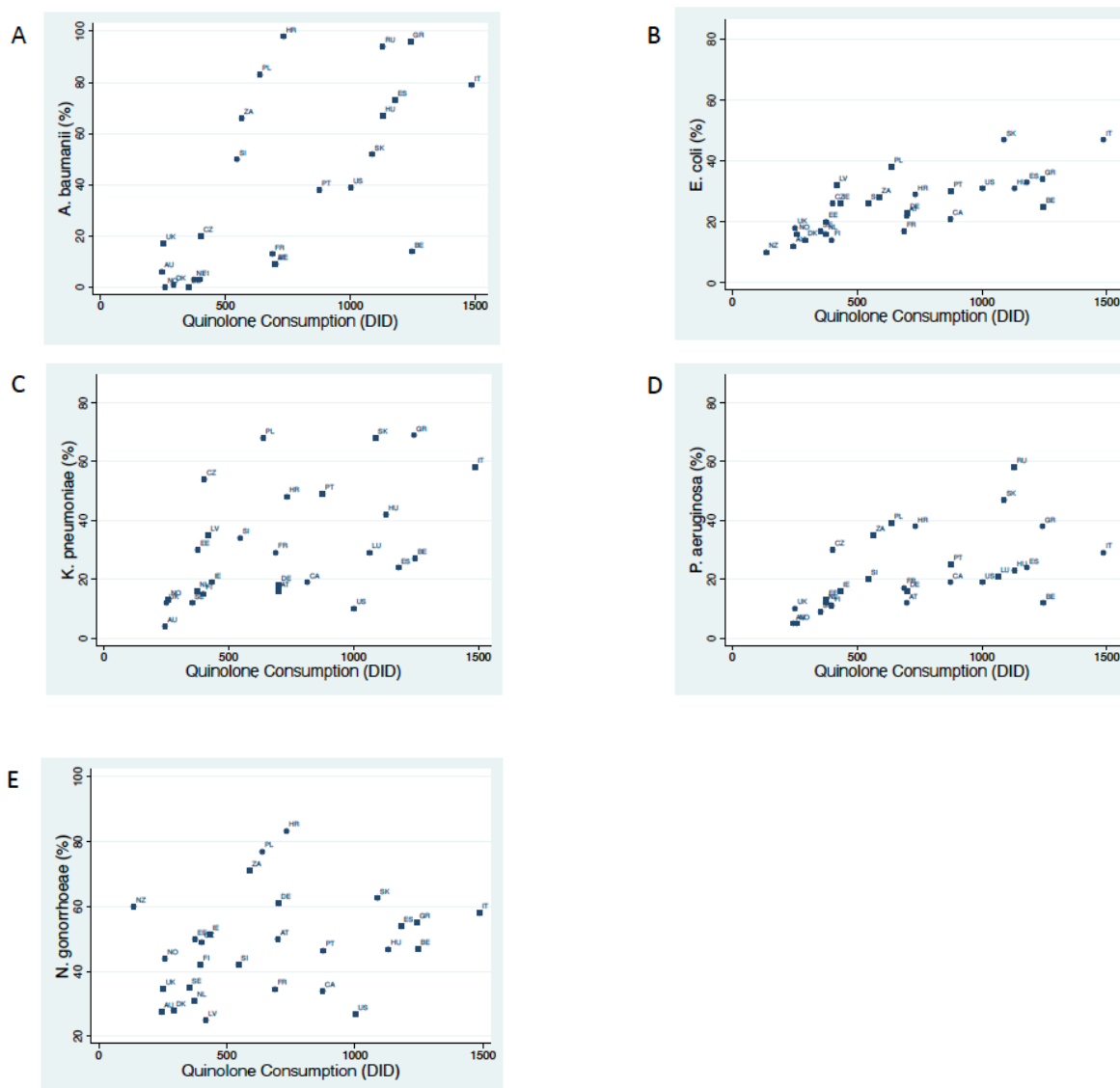
Abbreviations: AT, Austria; BE, Belgium; CN, China; CZ, Czech Republic; DE, Germany; DK, Denmark; EE, Estonia; EL, Greece; ES, Spain; FI, Finland; FR, France; HR, Croatia; HU, Hungary; IE, India, IN; Ireland; IT, Italy; LU, Luxembourg; LV, Latvia; NL, the Netherlands; NO, Norway; PL, Poland; PT, Portugal; SE, Sweden; SI, Slovenia; SK, Slovakia; UK, United Kingdom; US, United States; VN, Venezuela

References

1.   Unemo M, Bradshaw CS, Hocking JS, de Vries HJC, Francis SC, Mabey D, et al. Sexually transmitted infections: challenges ahead. Lancet Infect Dis. 2017;17(8):e235-e79. Epub 2017/07/14. doi: 10.1016/S1473-3099(17)30310-9. PubMed PMID: 28701272.

2.   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.

3.   Lankford C. The In Vitro Tolerance of the Gonococcus for Penicillin. American Journal of Syphilis. 1945;29(1):56-63.

4.   Del Love B, Finland M. Susceptibility of Neisseria gonorrhoeae to Eleven Antibiotics and Sulfadiazine. Comparison of Susceptibility of Recently Isolated Strains with Results obtained in Previous Years in the Same Laboratory. Archives of Internal Medicine. 1955;95(1):66-73.

5.   Willcox RR. A survey of problems in the antibiotic treatment of gonorrhoea. With special reference to South-East Asia. Br J Vener Dis. 1970;46(3):217-42. Epub 1970/06/01. doi: 10.1136/sti.46.3.217. PubMed PMID: 4246923; PubMed Central PMCID: PMCPMC1048070.

6.   Tedijanto C, Olesen SW, Grad YH, Lipsitch M. Estimating the proportion of bystander selection for antibiotic resistance among potentially pathogenic bacterial flora. Proc Natl Acad Sci U S A. 2018;115(51):E11988-E95. Epub 2018/12/19. doi: 10.1073/pnas.1810840115. PubMed PMID: 30559213; PubMed Central PMCID: PMCPMC6304942.

7.   Fernandez-Huerta M, Barbera MJ, Serra-Pladevall J, Esperalba J, Martinez-Gomez X, Centeno C, et al. Mycoplasma genitalium and antimicrobial resistance in Europe: a comprehensive review. Int J STD AIDS. 2020:956462419890737. Epub 2020/02/01. doi: 10.1177/0956462419890737. PubMed PMID: 32000587.

8.   Kenyon C. Prevalence of macrolide resistance in Treponema pallidum is associated with macrolide consumption. Journal of Medical Microbiology. 2018;Feb;68(2):119-123. doi: 10.1099/jmm.0.000885. Epub 2018 Dec 6.

9.   Kenyon C, Buyze J, Wi T. Antimicrobial consumption and susceptibility of Neisseria gonorrhoeae: a global ecological analysis. Frontiers in medicine. 2018;5:329.

10.   Kenyon C, Buyze J, Spiteri G, Cole M, Unemo M. Population-level antimicrobial consumption is associated with decreased antimicrobial susceptibility in Neisseria gonorrhoeae in 24 European countries: an ecological analysis. The Journal of infectious diseases. 2019;[Epub ahead of print]. Epub Apr 8. doi: 10.1093/infdis/jiz153.

11.   Olesen SW, Torrone EA, Papp JR, Kirkcaldy RD, Lipsitch M, Grad YH. Azithromycin Susceptibility Among Neisseria gonorrhoeae Isolates and Seasonal Macrolide Use. J Infect Dis. 2019;219(4):619-23. Epub 2018/09/22. doi: 10.1093/infdis/jiy551. PubMed PMID: 30239814; PubMed Central PMCID: PMCPMC6350947.

12.   Kirkcaldy RD, Bartoces MG, Soge OO, Riedel S, Kubin G, Del Rio C, et al. Antimicrobial Drug Prescription and Neisseria gonorrhoeae Susceptibility, United States, 2005-2013. Emerg Infect Dis. 2017;23(10):1657-63. Epub 2017/09/21. doi:

10.3201/eid2310.170488. PubMed PMID: 28930001; PubMed Central PMCID: PMCPMC5621530.

13.   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.

14.   The Center for Disease Dynamics Economics & Policy. ResistanceMap: Antibiotic resistance. 2021 [cited 2021]. Available from: 2021. https://resistancemap.cddep.org/AntibioticResistance.php.

15.   Limmathurotsakul D, Sandoe JA, Barrett DC, Corley M, Hsu LY, Mendelson M, et al. 'Antibiotic footprint'as a communication tool to aid reduction of antibiotic consumption. J Antimicrob Chemoth. 2019;74(8):2122-7.

16.   Kenyon C, Manoharan-Basil SS, Van Dijck C. Is There a Resistance Threshold for Macrolide Consumption? Positive Evidence from an Ecological Analysis of Resistance Data from Streptococcus pneumoniae, Treponema pallidum, and Mycoplasma genitalium. Microb Drug Resist. 2021. Epub 2021/02/18. doi: 10.1089/mdr.2020.0490. PubMed PMID: 33596133.

17.   Allan-Blitz L-T, Humphries RM, Hemarajata P, Bhatti A, Pandori MW, Siedner MJ, et al. Implementation of a rapid genotypic assay to promote targeted ciprofloxacin therapy of Neisseria gonorrhoeae in a large health system. Clinical infectious diseases. 2017;64(9):1268-70.

18.   Eyre DW, Sanderson ND, Lord E, Regisford-Reimmer N, Chau K, Barker L, et al. Gonorrhoea treatment failure caused by a Neisseria gonorrhoeae strain with combined ceftriaxone and high-level azithromycin resistance, England, February 2018. Eurosurveillance. 2018;23(27):1800323.

19.   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.

20.   Klein EY, Van Boeckel TP, Martinez EM, Pant S, Gandra S, Levin SA, et al. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. Proc Natl Acad Sci U S A. 2018;115(15):E3463-E70. Epub 2018/03/28. doi: 10.1073/pnas.1717295115. PubMed PMID: 29581252; PubMed Central PMCID: PMCPMC5899442.

21.   Kenyon C, De Baetselier I, Wouters K. Screening for STIs in PrEP cohorts results in high levels of antimicrobial consumption. Int J STDs & AIDS. 2020.

22.   Lewis DA. The role of core groups in the emergence and dissemination of antimicrobial-resistant N gonorrhoeae. Sex Transm Infect. 2013;89 Suppl 4:iv47-51. doi: 10.1136/sextrans-2013-051020. PubMed PMID: 24243880.

23.   Chyou P-H. Patterns of bias due to differential misclassification by case–control status in a case–control study. European journal of epidemiology. 2007;22(1):7.

24.   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).

25.   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.