Published in final edited form as:
Sex Transm Dis. 2012 May ; 39(5): 332–334. doi:10.1097/OLQ.0b013e318248a85f.

Emergence of Fluoroquinolone Resistance in *Neisseria gonorrhoeae* Isolates from Four Clinics in Three Regions of Kenya

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Abstract

We have recently reported high levels of fluoroquinolone resistance in a single region of Kenya. In this manuscript, we report high prevalence of fluoroquinolone resistance (53.2%) in *Neisseria gonorrhoeae* isolates from four clinics in three additional regions of Kenya. These findings highlight the need to change first-line treatment in these settings and the need to evaluate empiric management guidelines for treatment of gonococcal infection in Kenya.

Keywords

Fluoroquinolone; Sexually transmitted disease; gonorrhoea; cefixime; azithromycin

Single dose cephalosporin therapy (e.g. cefixime or ceftriaxone) is the recommended empiric treatment for gonococcal infections in most guidelines from industrialized countries [1, 2]. Furthermore, although the 2003 World Health Organization (WHO) guidelines for the management of gonococcal infection include single dose fluoroquinolone therapy unless prevalence of resistance exceeds an arbitrary threshold of 5%, recent publications by the WHO have recognized that fluoroquinolone and multi-drug resistant *Neisseria gonorrhoeae* is an emerging problem worldwide [3, 4]. Current treatment guidelines for syndromic management of urethritis and cervicitis in Kenya recommend single 800 mg dose norfloxacin for treatment of gonococcal infection (Guidelines available at the following URL: http://collections.infocollections.org/whocountry/en/d/Jh4329e/). However, the prevalence of fluoroquinolone resistance in Kenya is largely unknown. Studies in the 1990s demonstrated universal susceptibility to fluoroquinolones [5, 6]. More recently, resistance has

been documented in western Kenya and a restricted area of coastal Kenya [7, 8]. The purpose of this study was to determine the prevalence of fluoroquinolone resistance among isolates recovered from high-risk populations in three geographically distinct regions of Kenya.

Isolates were collected in 2009 and 2010 from four sites: Kisumu in western Kenya, the capital city Nairobi located on the principal highway connecting Mombasa and Kisumu, and Mombasa and Kilifi in coastal Kenya. Isolates were collected from high-risk individuals attending community or research clinics for medical care which included screening and management of sexually transmitted infections. Comparable data collection across sites included age, gender, HIV status, sex work, and circumcision status of males. When clinically indicated, patients had urethral or cervical swabs collected that were cultured directly to modified Thayer-Martin medium. Cultures were incubated in 5% CO_2 at 36°C up to 48 hours. *Neisseria gonorrhoeae* was identified using colonial morphology, Gram-stain, oxidase and the superoxol test.

Susceptibility testing to cefixime, ceftriaxone, azithromycin, ciprofloxacin and/or norfloxacin was performed using antibiotic disk diffusion or epilometry (E-test, BioMerieux, Marseille, France) on GC base agar (Oxoid, Basingstoke, UK) with 1% IsoVitalex (Oxoid) supplement. Antimicrobials studied and methods (disk or E-test) varied slightly at each site (Table 1). Quality control of the medium and antimicrobials was assured using *N. gonnorhoeae* strain ATCC 49226. Where available, interpretative breakpoints for disk diffusion and episilometry assays were taken from the Clinical Laboratory Standards Institute (CLSI) document M100-S21. Because CLSI breakpoints were not available for azithromycin or norfloxacin, we used the breakpoints established by Knapp *et al.* [9] (norfloxacin) and Mehaffey *et. al.* [10] (azithhomycin). For these, the disk diffusion resistance breakpoints used in this study were: azithromycin ≤ 24mm and norfloxacin ≤32 mm and for epsilometry and breakpoint for azithromycin resistance was ≥ 2μg/mL.

For statistical analysis and data presentation, isolates were considered either resistant or susceptible. Where applicable (ciprofloxacin only) isolates with intermediate susceptibilities according to CLSI breakopoints were included in the susceptible group. Differences in regional resistance and association between demographic data and resistance were evaluated using Fisher's exact test. Statistical analysis was performed using JMP version 9.0 (SAS, Cary, NC).

A total of 154 single isolates from 82 females and 72 males were tested: 64 from Kisumu, 44 from Nairobi, 29 from Mombasa and 17 from Kilifi (Table 1). The 17 isolates from Kilifi have been previously reported [8], but are included here to demonstrate that the new findings are consistent with the earlier reported resistance rates. Susceptibility testing (Table 1) demonstrated that overall prevalence of resistance to fluoroquinolones was 53.2% (95% CI, 45.3–61.8%). No significant difference in fluoroquinolone resistance was noted between the sites, or by patient characteristics. No resistance was observed for cefixime or ceftriaxone. Seven (6.5%) isolates had either azithromycin MICs of 0.5 μg/mL (n = 3) or inhibition zone diameters of between 25 and 28 mm (n = 4).

Fluoroquinolone resistance in *Neisseria gonorrhoeae* has been documented in North and South America, Europe, Southeast and South Asia and Australia [8, 11]. Although fluoroquinolone therapy was considered first line therapy for treatment of gonorrhoea for a number of years, most national guidelines from industrialized countries now discourage their use because of high resistance rates [1, 2]. Increased MICs to cefixime and other cephalosporins (but still considered susceptible) have also been documented in a number of countries and prevalence appears to be increasing, particularly in Asia and the Western Pacific Region [2, 11, 12]. Some of these isolates have been associated with treatment failures

when single dose oral cephalosporins are used to treat the infection [4, 11]. More recently, *N. gonorrhoeae* isolates with *in vitro* resistance to cephalosporins, including ceftriaxone, and treatment failures with ceftriaxone therapy have been reported [13, 14]. The potential emergence of multi-drug resistant *N. gonorrhoeae* underscores the urgency of ongoing surveillance for timely detection and response.

Evaluation of antimicrobial resistance in *N. gonorrhoeae* isolates from Kenya has been sporadic over the past 40 years. Studies in the 1970s demonstrated high rates of penicillin resistance [15, 16] and subsequent studies in the 1980s documented high rates of tetracycline resistance [6, 17, 18]. Studies conducted in the 1990s revealed high rates of penicillin and tetracycline resistance with no fluoroquinolone resistance observed [5, 6]. No further antimicrobial susceptibility studies were published until 2009 when Mehta *et al.* reported a high prevalence of fluoroquinolone resistance [7]. The current study confirms the presence of fluoroquinolone resistance in selected high-risk populations in Kisumu, Nairobi, Mombasa, and Kilifi. The cause of emergence of resistance is unclear. Possible explanations include progressive accumulation of Quinolone-Resistant Determining Region (QRDR) mutations leading to resistance, or the spread of a drug resistance clone introduced into Kenya leading to more rapid emergence such as that which has recently occurred in South Africa [19]. As with penicillin and tetracycline, it is likely that generalized overuse of fluoroquinolones has contributed to the emergence of resistance. Fortunately, the current study identified neither macrolide resistance nor increased cefixime MICs, suggesting that these agents should be clinically effective at the present time in the populations studied.

We identified 7 isolates with azithromycin MICs of 0.5μg/mL or inhibition diameters between 25 and 28 mm. Although these isolates are considered susceptible by the breakpoints suggested by Mehaffey *et al.* [10], isolates with an azithromycin MIC of 0.5μg/mL are considered “intermediately” susceptible according to European breakpoints (available at www.eucast.org). Furthermore, British susceptibility guidelines (available at www.bsac.org.uk) indicate that isolates with azithromycin inhibition zone diameters less than 28 mm should be reported as resistant. Although the implication of finding such isolates in our study is unclear, treatment failures have been reported in patients treated with a 1 gram dose of azithromycin who had been infected with such isolates [20].

Our study had limitations. The isolates studied do not represent a random sample. Rather, specimens were collected from individuals attending research clinics. Considering the estimated 2 – 3% prevalence rate of gonorrhoea in sub-Saharan Africa [12] our numbers constitute a very small proportion of the total number of cases in Kenya. In three sites, patients were nearly exclusively high-risk sex workers. Participants in these cohorts may have been exposed to higher rates of STI screening and treatment than the general population, which may have influenced the proportion of isolates with fluoroquinolone resistance. As such, these results may not reflect the resistance rates in the general population. Nonetheless, the findings remain striking, and raise concern that widespread resistance to fluoroquinolones could already be present in Kenya. The high resistance rates observed in the symptomatic men without defined risk factors from Kisumu also suggests that high resistance rates may extend beyond high-risk cohorts.

The high level of resistance to fluoroquinolones in *N. gonorrhoeae* isolates from a range of higher risk populations in different regions of Kenya is worrisome. These data suggest that recommended regimen for first-line treatment of gonorrhoea should be changed to cefixime or a recognized alternative cephalosporin in high-risk populations. All participating clinics are now using cefixime to treat gonorrhoea. Careful consideration should be given to changing the recommended regimen in other populations as well. If fluoroquinolones are used as first-line treatment, either because of lack of data unavailability of alternatives,

clinicians and patients should be aware of the risk for resistance and treatment failure. Revision of the national treatment guidelines should be considered and changes guided by collection of additional data on isolates from a sampling of all regions in Kenya. Ongoing surveillance will be crucial to follow the rates of fluoroquinolone resistance in the general population, characterize possible geographic variations in a larger sample, and monitor susceptibility to cephalosporins and azithromycin.

Acknowledgments

Kilifi: Salim Mwarumba, Benedict Mvera, and Susan Morpeth at the Kenya Medical Research Institute, Kilifi. The International AIDS Vaccine Initiative (IAVI) for supporting the high-risk cohort studies in Kilifi. This report was published with permission from KEMRI.

Mombasa: NIH grants R01 AI58698 and P01 HD64915. *The contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.*

Nairobi: Nancy Laing at the Health Sciences Centre, Winnipeg, Canada and Lisa Avery and Maryanne Crockett at the University of Manitoba, Jeckoniah N'dinya-Achola at the University of Nairobi.

Kisumu: Ruth Murugu and Lawrence Agunda, Partners in Reproductive Health, Kisumu, Kenya.

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TABLE 1

Number (percent) of isolates resistant to antimicrobials at each site.

Antimicrobial	Testing method	Fluoroquinolone[a]	Ceftriaxone	Cefixime	Azithromycin
Nairobi (n=44)	E-test	21 (47.7)	0 (0)	0 (0)	0 (0)
Kisumu (n=64)	Disk diffusion	34 (53.1)	0 (0)	0 (0)	0 (0)
Mombasa (n=29)	Disk diffusion	16 (55.2)	0 (0)	N/A[b]	N/A
Kilifi (n=17)	Disk diffusion[c] E-test™	11 (64.7)	0 (0)	N/A	N/A
All sites (n=154)		82 (53.2)	0 (0)	0 (0)	0 (0)

[a] Resistant to norfloxacin and/or ciprofloxacin

[b] Not done

[c] Disk diffusion performed for ceftriaxone and E-test™ for ciprofloxacin