NEISSERIA GONORRHOEAE ANTIMICROBIAL RESISTANCE SURVEILLANCE IN GAUTENG PROVINCE, SOUTH AFRICA

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Introduction

Gonorrhoea is a sexually transmitted disease caused by the bacterium *Neisseria gonorrhoeae*. Although many infected persons are asymptomatic, gonorrhoea is a major public health concern worldwide. The infection has a short incubation period lasting a few days, as well as a high transmission efficiency, and leads to a fivefold increase in HIV transmission and complications such as pelvic inflammatory disease and infertility which compound the global health burden.[1] The WHO 2012 prevalence data for curable sexually transmitted infections revealed that the estimated global prevalence of gonorrhoea among women aged 15-49 years was 0.8% (95% uncertainty interval 0.6-1.0%) ; and among 15-49 year old males 0.6% (0.4-0.9%).[2] These estimates corresponded to 78 million (53-110 million) new cases of gonorrhoea.

Neisseria gonorrhoeae, an obligate human pathogen, has displayed an alarming propensity to acquire resistance, through genetic mechanisms (both chromosomal and plasmid-mediated), to all sequential first-line antimicrobial agents used over the years.[3] Penicillin was first used in the 1940s and tetracycline from the 1950s - 1980s, but high-level plasmid-mediated resistances to both agents were being described by the 1980s. Quinolones were introduced in the early 1980s but resistance emerged in the Asia-Pacific region and then spread globally.[4] Antimicrobial resistance does not appear to confer a fitness cost as resistant strains predominate globally following withdrawal of the antimicrobial in question from clinical use.[4]

Extended-spectrum cephalosporins (ESCs) are considered to be the last antimicrobial class suitable for widespread single-dose, single-agent treatment. Cefixime is the only oral ESC that meets the criteria for the effective treatment of pharyngeal gonorrhoea with a > 95% cure rate. In Japan in the 1990s, use of a variety of oral ESCs with suboptimal efficacies in inadequate dosing regimens led to ultimate treatment failure with cefixime.[5] By 2010, clinically confirmed treatment failures had been described in Europe and North America.[6]

The WHO Global Gonococcal Antimicrobial Surveillance Program (GASP) was relaunched in 2009 to monitor the trends of antimicrobial resistance in *N. gonorrhoeae* and improve knowledge on potential resistance mechanisms through laboratory testing.[1] South Africa is a participating country, and the Sexually Transmitted Infections (STI) laboratory of the Centre for HIV and STIs (CHIVSTI) of the NICD is a regional institution for the GASP network in WHO-Africa.

Data showing the distribution of STI syndromes among males and females attending primary healthcare facilities (PHCs) in South Africa reveal that Male Urethritis Syndrome (MUS) and Vaginal Discharge Syndrome comprise the bulk of STI presentations.[7] Periodic aetiological surveillance of STI syndromes is essential to update and validate the existing syndromic management guidelines. The Centre for HIV and STIs has co-ordinated microbiological surveillance in patients

presenting to sentinel PHCs since 2005. Results indicate that *N. gonorrhoea* is the predominant cause of MUS (70-80%) and is present in 10-15% of symptomatic VDS cases.[8] Antimicrobial susceptibility testing of *N. gonorrhoeae* isolates forms an integral part of this aetiological surveillance. Resistance surveys have been conducted annually in Gauteng since 2007, and periodically in other provinces.

Resistance patterns and trends to various antimicrobials in *N. gonorrhoeae* isolates from STI surveillance in Gauteng Province over an eight-year period spanning 2008 to 2015 are described here.

Methods

Neisseria gonorrhoeae was cultured from swab specimens of genital discharge (endocervical and urethral) in consenting adult patients. From 2007-2014, national microbiological surveillance activities were undertaken by the provincial Department of Health. This required the systematic recruitment of consecutive consenting adult patients (both male and female) presenting with genital discharge to each sentinel PHC. In 2015, STI surveillance was incorporated into the NICD GERMS-SA surveillance platform. Surveillance activities involved sampling of approximately 150-200 males presenting with urethritis for the isolation of at least 100 viable gonococcal isolates per site.

Specimens were either directly inoculated onto specialised agar and the culture plates were placed in candle jars for transfer to the NICD STI laboratory, or swabs were placed in Amies™ transport media and transported on ice for culture in the reference laboratory. At the laboratory, gonococcal isolates were tested for susceptibility to antimicrobials by E-test™ (cefixime, ceftriaxone, ciprofloxacin) or agar dilution (penicillin, tetracycline, azithromycin) according to established standard operating protocols. Minimum inhibitory concentrations (MICs) were interpreted according to criteria recommended by the Clinical Laboratory Standards Institute (CLSI)[9], or for azithromycin susceptibility, according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints.[10] For purposes of quality assurance a panel of control strains of *N. gonorrhoeae* with known MICs to each antimicrobial was included in every batch of clinical isolates tested.

Data analysis: Analysis of susceptibility trends was performed using Stata™ version 14 for *Neisseria gonorrhoeae* isolates obtained from annual Gauteng surveillance at a single sentinel site between 2008 and 2015. The relative prevalence of susceptibility and resistance was determined for those antimicrobials to which resistance is well-established. Chi-square tests were used to determine whether the difference in high-level resistance prevalence between calendar years reached statistical significance.

For antimicrobials currently recommended for use, MIC50 (minimum concentration needed to inhibit 50% of isolates); MIC90 (minimum concentration needed to inhibit 90% of isolates); and maximum MICs were determined by year. The K-sample test was used to test for equality of medians (MIC50) across calendar years, while linear regression and likelihood-ratio tests were used to test for MIC trends.

Results

Resistance profiles of N. gonorrhoeae to antimicrobials tested by calendar year: Neisseria gonorrhoeae isolates for E-test MIC were available for every consecutive year from 2008-2015 (Table 1). Fewer isolates were tested using agar dilution, and data are available from 2011-2015 (Table 1). Agar dilution MIC testing was not performed in the 2014 calendar year.

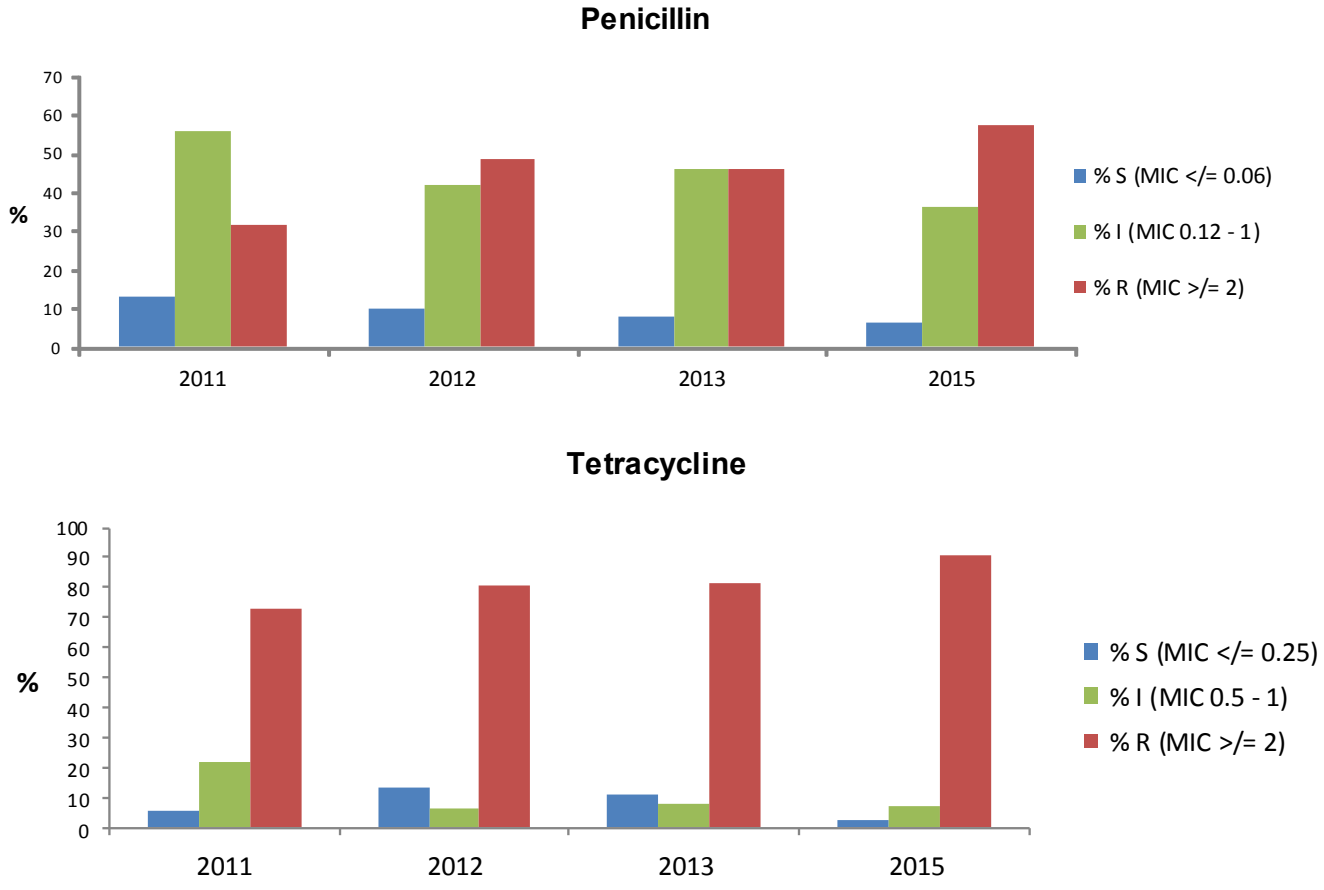
Table 1: Numbers of *Neisseria gonorrhoeae* isolates used for antimicrobial susceptibility testing (AST) by calendar year for the period 2008 to 2015, Gauteng, South Africa.

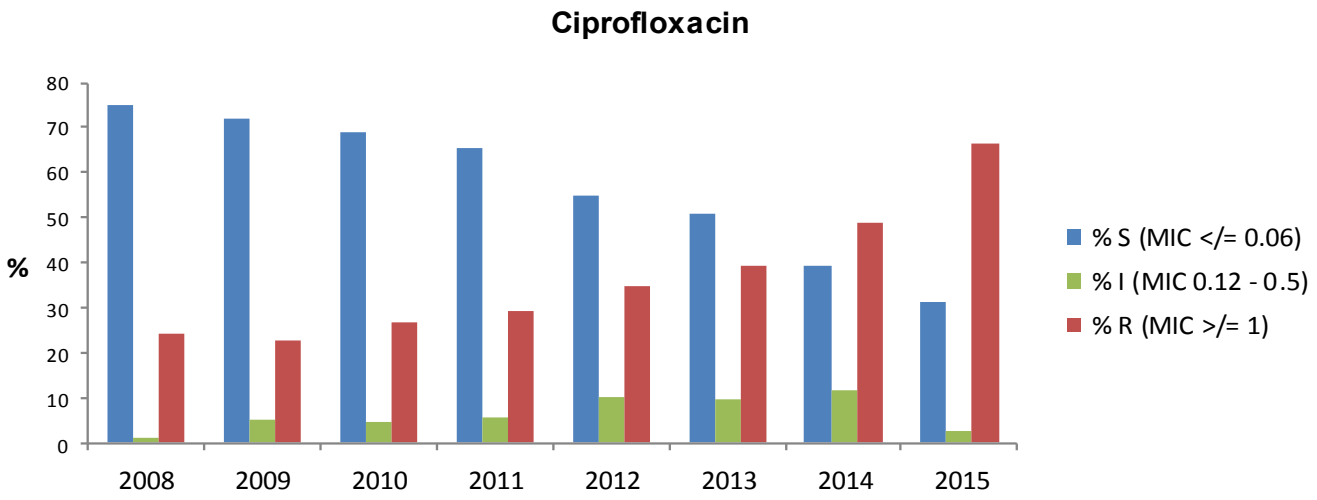
Year	Antimicrobials & AST Method Cefixime (CXM), Ceftriaxone (CTR), Ciprofloxacin (CIP) E-test MIC	Azithromycin (AZT), Penicillin (PEN), Tetracycline (TET) Agar dilution MIC
2008	328 (CTR & CIP only)	
2009	324	
2010	316	
2011	282	70
2012	294	31
2013	228	78
2014	205	
2015	135 (CXM & CTR only)	125 (TET; PEN; CIP; AZT)

MIC = minimum inhibitory concentration

During the periods under review, there was a general increase in the prevalence of *N. gonorrhoeae* resistance to penicillin, tetracycline and ciprofloxacin (Figure 1). Between 2011 and 2015, the prevalence of high-level resistance rose from 31% to 57% for penicillin (p=0.009) and from 73% to 91% for tetracycline (p=0.009). Between 2008 and 2015, the prevalence of high-level resistance to ciprofloxacin rose exponentially from 24% to 67% (p <0.001).

Figure 1: Antimicrobials to which resistance was established by calendar year, Gauteng, South Africa.





S = Susceptible; I = Intermediately-resistant; R = resistant; MIC = minimum inhibitory concentration

The Clinical Laboratory Standards Institute defines decreased susceptibility to extended-spectrum cephalosporins (DS ESC) as a MIC ≥ 0.5 µg/ml[7]; whereas EUCAST uses a cut-off that is one double-dilution lower at ≥ 0.25 µg/ml.[7] More than 99% of isolates were especially sensitive to ESCs (Table 2). Decreased susceptibility to cefixime was not observed using CLSI interpretive criteria, whereas it was seen in one isolate from 2013 (0.4%) using EUCAST cut-offs. Two isolates from 2009 exhibited decreased susceptibility to ceftriaxone using EUCAST criteria (0.6%) and one of these (0.3%) also showed reduced ceftriaxone susceptibility according to CLSI breakpoints (Table 3). Unfortunately, these two isolates were not available for further analysis.

Trend analysis revealed an MIC creep for cefixime, i.e. a significant increase in MIC50 and MIC90, notably in 2015 (Table 2).

Table 2: Minimum inhibitory concentration (MIC) trend analyses for cefixime, Gauteng, South Africa, 2008-2015.

Year	No of isolates	MIC_{50}	MIC_{90}	Maximum MIC	% with MIC = 0.125	% with MIC = 0.25	% with MIC >/= 0.5
2009	324	<0.016	0.016	0.064	0.00	0	0.0
2010	316	<0.016	<0.016	0.016	0.00	0	0.0
2011	282	<0.016	<0.016	0.016	0.00	0	0.0
2012	294	<0.016	<0.016	0.016	0.00	0	0.0
2013	228	<0.016	0.016	0.25	0.00	0.4 (1)	0.0
2014	205	<0.016	0.016	0.047	0.00	0	0.0
2015	125	0.016	0.032	0.064	0.00	0	0.0

p-value for equality of medians across years < 0.001

Table 3: Minimum inhibitory concentration (MIC) trend analyses for ceftriaxone, Gauteng, South Africa, 2008-2015

Year	No of isolates	MIC_{50}	MIC_{90}	Maximum MIC	% with MIC = 0.125	% with MIC =0.25	% with MIC >/= 0.5
2008	328	0.002	0.004	0.008	0	0	0
2009	324	0.003	0.006	0.38	0	0.3 (1)	0.3 (1)
2010	316	0.002	0.006	0.032	0	0	0
2011	282	0.003	0.004	0.012	0	0	0
2012	294	0.003	0.004	0.016	0	0	0
2013	197	0.003	0.006	0.064	0	0	0
2014	205	0.004	0.008	0.016	0	0	0
2015	135	0.003	0.006	0.023	0	0	0

The Clinical Laboratory Standards Institute has not established interpretive criteria for azithromycin. EUCAST defines resistance as MIC > 0.5 µg/ml, based on an epidemiological cut-off of 1 µg/ml for wild-type *N. gonorrhoeae* isolates.[10] The US Gonococcal Isolate Surveillance Project (GISP) uses a breakpoint of $\geq$ 2 µg/ml to define elevated MICs to azithromycin and increased likelihood of treatment failure.[6]

Elevated azithromycin MICs were observed in 2013 and 2015: in 10/78 isolates (13%) and 5/125 isolates (4%), respectively, according to EUCAST criteria (Table 4). Further analysis, including repeat agar-dilution MIC testing, will be undertaken to determine reproducibility of these results and whether these isolates represent a single clone. Isolates with MIC $\geq$ 2 µg/ml, and defined as having reduced susceptibility based on the GISP surveillance case definition, were detected only in 2013 (2/78; (3%)).

Table 4: Minimum inhibitory concentration (MIC) trend analyses for azithromycin, Gauteng, South Africa, 2011-2015

Year	No. of isolates	MIC_{50}	MIC_{90}	Maximum MIC	% with MIC </= 0.25	% with MIC > 0.5	% with MIC >/= 2
2011	70	0.128	0.25	0.5	93	0.0	0.0
2012	31	0.128	0.5	0.5	87	0.0	0.0
2013	78	0.25	1	4	72	13 (10)	3 (2)
2015	125	0.25	0.5	1	89	4 (5)	0.0

p-value for equality of medians across years < 0.001

Discussion

These data reveal that penicillin and tetracycline are unlikely to be included in any future genital discharge treatment algorithms in South Africa. In South African isolates, high-level penicillin resistance was found to be plasmid-mediated i.e. a novel beta-lactamase producing "Johannesburg" plasmid was identified and these penicillinase-producing isolates were discovered to be clonally related.[11] Similarly, two types of tetracycline resistant *N. gonorrhoeae* (TRNG) plasmids have been

detected and they confer high-level resistance to the drug.[12]

Escalating ciprofloxacin resistance was seen in Johannesburg and Cape Town when data from 2004 and 2007 were compared.[13] In Johannesburg, there was a 2.9-fold increase in resistance prevalence from 11% to 32%; and in Cape Town a 3.8-fold increase from 7% to 27%. The World Health Organization recommends a change of empirical treatment for gonorrhoea when the resistance threshold reaches 5%.[14] The South African syndromic management guidelines were therefore formally revised in 2008 to replace ciprofloxacin and incorporate cefixime as first-line therapy for gonorrhoea.[15]

The primary resistance determinant to extended-spectrum cephalosporins (ESCs) is a specific alteration in the *penA*gene encoding penicillin binding protein 2 (PBP2). This occurs through acquisition and recombination into its genome of foreign gene sequences from commensal *Neisseria* species residing in the oropharynx. This transformation gives rise to a mosaic *penA* gene encoding a mosaic PBP2 with reduced target affinity for ESCs: the MICs of cefixime are increased proportionately more than those of ceftriaxone.[16] In South Africa, in 2012, the first two cases of DS ESC associated with cefixime treatment failure were described in two patients presenting with persistent urethral discharge.[17] Genetic characterization of the two isolates using *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST) and multi-locus sequence typing (MLST), revealed identical sequence types which represent a multi-drug resistant clone characterized by DS ESC and global spread. Both patients were in the men-who-have-sex-with-men (MSM) risk group. There are two factors that could lead to the spread of resistance in this key population: high risk sexual behaviour and participation in international sexual networks, and the presence of pharyngeal gonorrhoea, which is typically asymptomatic. Gonococci residing in the pharynx are at a survival advantage due to differential concentrations of antimicrobials at this site, and the opportunity for DNA exchange with oropharyngeal commensal *Neisseria* species. An additional two cases of DS to cefixime were identified in MSM residing in Cape Town and East London, respectively (D. Lewis, unpublished data).

In 2009, the world's first confirmed extensively-drug resistant (XDR) *N. gonorrhoeae* infection was reported from Japan.[18] The gonococcal strain, isolated from the pharynx of a female sex worker (FSW), displayed high-level resistance to both cefixime (MIC = 8) and ceftriaxone (MIC = 2-4). It was also resistant to several other classes of antimicrobials, including fluoroquinolones, macrolides and tetracycline. Following these reports and in accordance with WHO recommendations, there was a national change in recommended first-line therapy for gonorrhoea from oral cefixime to injectable ceftriaxone (250mg single-dose) in 2014 in South Africa. Additional dual therapy with oral azithromycin (1g stat) was recommended.[19] This was a pro-active and pre-emptive approach, endorsed by the WHO, to limit the emergence of XDR *N. gonorrhoeae* worldwide, particularly in areas where there is a general lack of surveillance in key populations, such as MSM and FSW.[20]

Although clinical effectiveness of azithromycin for urethral and endocervical infections has been estimated to be >95%,[21] it is recommended only in dual therapy due to the ease of resistance development to macrolide monotherapy. Resistance has been described even with use of higher dose (2g) azithromycin monotherapy,[22] and this dose is associated with increased gastro-intestinal side-effects.

Failure of dual ceftriaxone-azithromycin therapy has recently been described, with persistence of pharyngeal gonorrhoea in a heterosexual man treated for urogenital symptoms.[23] The patient was infected with an XDR strain, which had acquired multiple resistance mechanisms to both ceftriaxone and azithromycin. Molecular epidemiology identified the isolate as MLST ST1901 and a new NG-MAST ST 12133, which belongs to a genogroup that is associated with extensive drug-resistance and is spreading in Japan.

This surveillance report describes resistance trends to various antimicrobials used in past and current gonorrhoea treatment regimens. It is limited by a lack of corresponding demographic and clinical data, including behavioural characteristics of patients presenting with genital discharge. Future analyses incorporating this information could be used to better understand transmission dynamics and inform control efforts. Additional analyses are planned using data from other provinces to study national trends in gonococcal antimicrobial resistance.

The ease with which *N. gonorrhoeae* develops drug resistance means that antimicrobial stewardship strategies are urgently needed. These should include rational, standardized and regulated prescription practice for genital discharge syndrome, as well as research and development initiatives for novel antimicrobials with unique mechanisms of action and their incorporation into appropriate therapeutic regimens.

There is a need for accurate rapid diagnostics that would facilitate screening for asymptomatic and extra-genital infection in high-risk and key population groups. Additionally, allocation of resources is required for enhanced local surveillance strategies. These would include theimplementation of activities designed to increase detection of treatment failure cases and extragenital (pharyngeal) infections at healthcare level, as well as sustained antimicrobial surveys (including test -of-cure using culture) in key populations.

Conclusions

Neisseria gonorrhoeae antimicrobial susceptibility profiles and trends for Gauteng over a period of eight years reveal that high-prevalence resistance to penicillin, tetracycline and ciprofloxacin obviates the use of these agents in empiric therapy guidelines for syndromic management.

The prevalence of resistance to ESCs is < 1%, validating continued use of ceftriaxone in dual therapy for gonorrhoea. However, it is essential that ESC and azithromycin susceptibility trends for representative numbers of isolates are monitored to detect emerging resistance timeously.

Key Points

- Antimicrobial resistance in *Neisseria gonorrhoeae* is increasing worldwide
- Extensively-drug resistant (XDR) strains are characterised by high extended-spectrum cephalosporin MICs
- Dual therapy with injectable ceftriaxone and azithromycin may curtail emergence of XDR strains
- Enhanced national and regional culture-based surveillance is essential to detect emerging resistance, particularly in key populations

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