

***Neisseria gonorrhoeae* isolates from four centres in Papua New Guinea remain susceptible to amoxicillin-clavulanate therapy**

PAMELA J. TOLIMAN¹, TONY LUPIWA^{1,2}, GREGORY J. LAW³, JOHN C. REEDER^{1,4} AND PETER M. SIBA¹

Papua New Guinea Institute of Medical Research, Goroka and Disease Control Branch, Division of Public Health, Papua New Guinea Department of Health, Port Moresby

SUMMARY

Antibiotic-resistant strains of *Neisseria gonorrhoeae* have the potential to undermine treatment and control of gonorrhoea, which remains a highly prevalent sexually transmitted infection (STI) in Papua New Guinea (PNG). The standard treatment regimen for gonorrhoea in PNG based on amoxicillin and clavulanic acid (amoxicillin-clavulanate) was introduced about 15 years ago and there is some concern that over time circulating strains may have developed resistance to this therapy. To investigate this, *N. gonorrhoeae* isolates (n=52) were collected from STI clinics in geographically representative centres in PNG and tested for their in vitro susceptibility to a range of antibiotics. All 52 isolates tested were found susceptible to amoxicillin-clavulanate, despite 40% (n=21) being penicillinase producers and thus resistant to penicillin. These findings indicate that amoxicillin-clavulanate therapy remains an effective treatment for gonococcal infections in PNG, and support the maintenance of the present standard treatment for gonorrhoea in PNG.

Introduction

Gonorrhoea, caused by the Gram-negative coccoid bacterium *Neisseria gonorrhoeae*, remains one of the most common sexually transmitted infections (STIs) in Papua New Guinea (PNG) (1-4). Rates have been steadily increasing over the last four decades (5) and the National Health Plan for 2001-2010 estimates the incidence rate for gonorrhoea to be 131 per 100,000 population (6). However, various studies have shown higher rates of gonococcal infection in selected populations within PNG and a survey of STI clinics reported that 54% of male attenders were clinically diagnosed with gonorrhoea (2). A high prevalence of gonococcal infection, detected by polymerase chain reaction (PCR), has also been reported among female sex workers in Port Moresby (34%), Lae (24%)

and the Eastern Highlands Province (21%) (1,4). Furthermore, in a seemingly asymptomatic rural population, gonococcal infection was detected in 18% of women (3).

While data on STIs in PNG are limited, the National Department of Health (NDoH) recently reported over 17,000 cases of genital discharge syndrome (GDS) nationally in the first half of 2008 alone (7). The large burden of STIs in PNG prompted the prioritization of STI management in the National Health Plan 2001-2010 and the PNG National Strategic Plan on HIV/AIDS [human immunodeficiency virus/acquired immune deficiency syndrome] 2006-2010 (6,8). Accordingly, the NDoH has made it a priority to improve STI case management through a syndromic management approach. Currently all cases of GDS, including vaginal discharge

¹ Papua New Guinea Institute of Medical Research, PO Box 60, Goroka, Eastern Highlands Province 441, Papua New Guinea

² Present address: National AIDS Council Secretariat, PO Box 1345, Boroko, National Capital District 111, Papua New Guinea

³ Disease Control Branch, Division of Public Health, National Department of Health, PO Box 807, Waigani, National Capital District 131, Papua New Guinea

⁴ Present address: Burnet Institute, GPO Box 2284, Melbourne, Victoria 3001, Australia

syndrome (VDS) and lower abdominal pain syndrome (LAPS) in women, and urethral discharge syndrome (UDS) in men, are treated for gonorrhoea, chlamydia and trichomoniasis in a single-dose therapy. This regimen consists of 1 g Augmentin 500, 2 g amoxicillin, 1 g probenecid and 1 g azithromycin with either 1 g tinidazole twice daily for three days (women) or 2 g tinidazole (men) (9). Augmentin 500 contains 500 mg of amoxicillin and 125 mg of clavulanic acid.

The treatment of gonorrhoea could potentially be undermined by the ability of the aetiological agent, *N. gonorrhoeae*, to develop and acquire antibiotic resistance genes (10,11). Thus, the monitoring of antibiotic resistance in *N. gonorrhoeae* is essential for informing and developing treatment guidelines within the country (12).

PNG has been a part of the World Health Organization Western Pacific Region Gonococcal Antimicrobial Surveillance Programme (WPR GASP) since 1993 (13). However, the source of PNG isolates tested in this program is unknown. In PNG, routine culture of *N. gonorrhoeae* is conducted only at the Port Moresby General Hospital (PMGH) (14), so there is concern that the PNG data included in the WPR GASP may not be representative of susceptibility patterns throughout the rest of the country. There is also a lack of data from either PMGH or WPR GASP on the susceptibility of gonococcal isolates to amoxicillin and clavulanic acid (amoxicillin-clavulanate), which is the basis of gonorrhoea treatment in the country (13,14). Furthermore, despite the importance of resistance monitoring, the most recent multicentre investigation into gonococcal antimicrobial susceptibility patterns in PNG was carried out well over fifteen years ago (2).

Amoxicillin-clavulanate has been used to treat gonorrhoea in PNG for the last 15 years, raising concern that isolates in current circulation may be developing resistance to therapy (9). To investigate this, we conducted surveillance of antibiotic resistance in *N. gonorrhoeae* in four major centres in PNG.

Methods

Study population and sample collection

This study was conducted between 2004

and 2005 at the STI clinics in Port Moresby, Lae, Mt Hagen and Goroka. During the study period, patients were invited to participate if they presented at the STI clinic with UDS (men), or VDS or LAPS (women). Eligible patients were asked to provide informed consent and then undergo a physical examination. A urethral swab was collected from men and an endocervical swab was taken from women. Ethics approval was obtained from the PNG Medical Research Advisory Committee.

Isolation of *N. gonorrhoeae*

The swab was used to inoculate GC (gonococcal) agar containing VCN (vancomycin, Colistin sulphate and nystatin) selective supplement (Oxoid Limited, Thebarton, SA, Australia) and chocolate agar. The inoculated media were placed in a candle jar (12-15% CO₂) and kept at room temperature until transported to the laboratory, where the media were streaked and incubated at 37°C in the candle jar for up to 48 hours.

Suspected colonies that grew within the 48-hour incubation period were presumptively identified as *N. gonorrhoeae* if they were Gram-negative diplococci, oxidase positive and superoxol positive. The rapid carbon utilization test as described by the WHO was used to confirm the identity of *N. gonorrhoeae* isolates (15).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of *N. gonorrhoeae* isolates was done using the disk diffusion method on Columbia chocolate agar as described by the WHO (15). The antibiotic discs (Oxoid Limited, Thebarton, SA, Australia) used contained amoxicillin and clavulanic acid in a 2:1 ratio (30 µg), azithromycin (15 µg), ceftriaxone (0.5 µg), ciprofloxacin (1 µg), erythromycin (15 µg), penicillin G (0.1 IU), spectinomycin (100 µg) and tetracycline (10 µg). The minimum inhibitory concentration (MIC) was measured for isolates that displayed a diminished susceptibility to an antibiotic using the Etest® system (AB Biodisk, Dalvågen, Solna, Sweden). Penicillinase-producing *N. gonorrhoeae* (PPNG) were identified using a β-lactamase indicator stick (Oxoid Limited, Thebarton, SA, Australia).

Results

Samples were collected from a total of 145 women and 65 men during the study period. *N. gonorrhoeae* was cultured from 31% (n=65) of patients : 14% (n=21) of women and 68% (n=44) of men. *N. gonorrhoeae* was isolated more commonly from urethral swabs of men than cervical swabs of women (odds ratio 12.4, $p < 0.001$). A total of 65 gonococcal strains were isolated, of which 52 (80%) underwent full antimicrobial susceptibility testing (Table 1).

21 isolates resistant to penicillin were PPNG and accounted for 40% of isolates tested. Of these, 7 isolates (33%) had an MIC of $\geq 32 \mu\text{g/l}$, the highest concentration on the penicillin Etest[®] strip used (Table 2). All isolates remained susceptible to amoxicillin-clavulanate, including the PPNG isolates. All non-PPNG (60%, n=31) were susceptible to penicillin (Table 1). One isolate (2%) from Lae was resistant to ciprofloxacin and had an MIC of $2 \mu\text{g/l}$. Elevated MICs to tetracycline were observed in 19% (n=10) of isolates tested (Table 2). All isolates collected in the study were susceptible to spectinomycin, erythromycin, azithromycin

and ceftriaxone (Table 1).

Discussion

All gonococcal isolates tested in this study were susceptible in vitro to amoxicillin-clavulanate. On the basis of this finding, the current treatment regimen of 1 g of Augmentin 500 with an additional 2 g of amoxicillin and 1 g of probenecid should remain the standard treatment for gonorrhoea, as part of the syndromic management protocol for GDS in PNG.

Similar to other studies in PNG (2,13,16,17), this study found that PPNG represented a large proportion (40%) of gonococcal isolates that cause disease in the community. PPNG are characteristically resistant to β -lactam antibiotics, because the penicillinase produced by PPNG hydrolyses the active component of β -lactam antibiotics, particularly penicillins (11,18). All PPNG detected in this study were resistant to penicillin, with high MICs to the antibiotic. However, all PPNG were susceptible to amoxicillin-clavulanate. Clavulanic acid, a β -lactamase inhibitor, blocks the activity of penicillinase making the organism susceptible

TABLE 1

ANTIMICROBIAL SUSCEPTIBILITY OF *NEISSERIA GONORRHOEAE* ISOLATES

		Port Moresby n=4	Lae n=18	Mt Hagen n=13	Goroka n=17	Total n=52
β -lactamase	Positive	2	6	5	8	21
Penicillin G	Resistant	2	6	5	8	21
Amoxicillin-clavulanate	Resistant	0	0	0	0	0
Ciprofloxacin	Resistant	0	1	0	0	1
Tetracycline	Resistant	0	0	0	0	0
Spectinomycin	Resistant	0	0	0	0	0
Erythromycin	Resistant	0	0	0	0	0
Azithromycin	Resistant	0	0	0	0	0
Ceftriaxone	Resistant	0	0	0	0	0

TABLE 2

NUMBER OF *NEISSERIA GONORRHOEAE* ISOLATES WITH ELEVATED MINIMUM INHIBITORY CONCENTRATIONS

Antimicrobial agent	MIC (µg/l)	Number of isolates
Penicillin	6	1
	8	3
	12	3
	16	4
	24	3
	32	7
Tetracycline	64	5
	96	4
	256	1
Ciprofloxacin	2	1

MIC = minimum inhibitory concentration

to amoxicillin (11,18). In spite of the persistence of PPNG in the community, the standard treatment for gonorrhoea remains effective and thus should be maintained.

Chromosomal-mediated resistance (CMR) to penicillin has been reported previously in PNG (2,16,19). CMR to penicillin is usually manifested in PPNG resistant to amoxicillin-clavulanate or with elevated MICs to penicillin in non-PPNG. CMR to penicillin was not detected in any of the isolates tested, as all isolates were susceptible to amoxicillin-clavulanate and all non-PPNG were susceptible to penicillin.

Resistance to ciprofloxacin and tetracycline have been reported in isolates from PNG (2,13,16,17). Quinolones, such as ciprofloxacin, are not commonly used to treat gonorrhoea in PNG and selective pressure from quinolone use would be minimal. The occurrence of quinolone resistance has been low and sporadic suggesting that quinolone-resistant strains may be introduced organisms, as their occurrence is widespread

throughout Asia and parts of the Pacific (13). In this study, one isolate (2%) was resistant to ciprofloxacin but remained susceptible to amoxicillin-clavulanate. Elevated MICs to tetracycline were observed in 19% of the isolates tested suggesting that the common use of doxycycline is selecting strains that are becoming resistant to tetracycline. While doxycycline is not used to treat gonorrhoea in PNG, it is occasionally used to treat chlamydia, pelvic inflammatory disease and associated sequelae (9).

Despite the shortcomings of culture *N. gonorrhoeae* was isolated from 31% of patients, suggesting that gonorrhoea remains a common STI in the country. As such, it is imperative that the standard treatment protocol be regularly monitored. To inform the development of future treatment protocols, susceptibility to a range of antibiotics must be regularly monitored in case the present standard treatment becomes ineffective (11,12). On the basis of our findings, spectinomycin,

erythromycin, azithromycin and ceftriaxone remain effective and could offer alternative options for gonococcal treatment in PNG.

The diversity of the terrain, people, language and culture in PNG give rise to complex transmission dynamics of STIs in the country. Hence it is vital that gonococcal susceptibility patterns be monitored across different sites within PNG. The four centres in this study were selected for their moderate to high incidence of gonococcal infection (8). Port Moresby, the capital and most populated city of PNG, is considered the gateway to the country. Lae, the second largest city, is also a strategic port and the start of the Okuk Highway, which links the coast to the Highlands Region. Mt Hagen and Goroka are major centres in the Highlands Region and are situated along the Okuk Highway. Obviously, a national standard treatment protocol should be effective throughout the entire country. Isolates collected across all four sites were susceptible to amoxicillin-clavulanate indicating that the standard treatment is still effective across PNG.

At present, culture-based methods are the gold standard for antibiotic susceptibility testing (11) but there are many challenges associated with the culture of *N. gonorrhoeae*, particularly in a resource-limited setting such as PNG. Firstly, *N. gonorrhoeae* is fastidious and difficult to culture, particularly from women because of the presence of competing flora in the female genital tract (20). The presence of competing flora might in part explain the significant difference in isolation of the organism from men compared to women. Secondly, most hospitals in PNG do not have the laboratory capacity and resources to carry out routine gonococcal culture. This is particularly important since successful culture of *N. gonorrhoeae* is dependent on many factors such as technique of specimen collection, transportation conditions, quality of culture medium and incubation conditions (20). The development of an assay that is able to detect relevant factors associated with resistance in *N. gonorrhoeae* without the need for a viable organism would allow a more representative monitoring of antibiotic susceptibility patterns throughout the country. Recently, a number of molecular assays have been developed to detect specific mutations associated with *N. gonorrhoeae* antimicrobial resistance using non-viable organisms (21,22). Such assays, perhaps at reference centres, should be

considered for future monitoring of gonococcal susceptibility in PNG.

Conclusion

Given the high prevalence of STIs in PNG, their effective treatment is important in itself but also an integral part of PNG's response to the HIV epidemic. Gonorrhoea, while easily curable, continues to be a significant STI in the country. Amoxicillin-clavulanate remains effective in clearing circulating strains of *N. gonorrhoeae* in PNG and therefore the current standard treatment regimen for gonorrhoea should be maintained. However, it is vital that gonococcal antibiotic susceptibility be regularly monitored so that the national treatment protocols continue to be evidence based.

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