

## Review Article

# The Plasmids in Gonococci

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### Introduction

Plasmids are the extra-chromosomal, double-stranded, circular DNA molecules found in bacteria occurring among both gram-positive and gram-negative variants. Although they are capable of replicating independently, plasmids can be integrated into the bacterial chromosome for replication and other functions.

Principally two different types of plasmids are found in the bacterial cells, both of which often exist in a single cell.<sup>1</sup>

(1) Cryptic plasmids: are small, non-transmissible plasmids having molecular weights 3-20 million Daltons and are frequently present in many (10-60) copies per cell. These plasmids do not contain genes for transfer from cell to cell.

(2) Transmissible plasmids: can transfer from cell to cell by conjugation via sex pili. They are large, having molecular weights 40-100 million Dalton and usually present in a few copies (one to three) per cell. They carry about a dozen of genes for synthesis of many structures like pili and functions including resistance to heavy metals and ultraviolet rays as well as to antibiotics. The plasmid-mediated antibiotic resistance is induced by a variety of enzymes, notably beta-lactamase and also for the enzymes required for their transfer.<sup>1</sup>

### Plasmid types in Gonococci:

Isolates of the *Neisseria gonorrhoeae* were found to harbour many different types of plasmids:

(i) Cryptic plasmids: Nearly all gonococci (about 96%) carry a cryptic plasmid of 2.6 MDa (approximately 4.2 kilobases) and are the smallest of all gonococcal plasmids. Functions of the cryptic plasmids are not known exactly.<sup>2</sup> The use of cryptic plasmid-derived probes for DNA hybridization was thought to have limited application for the detection of gonococci in clinical isolates. It has recently been shown that the polypeptide product of the *cpxB* gene of the cryptic plasmid *pJDI* was also produced by the plasmid-free strains and a copy of this gene was present in gonococcal chromosome of all strains tested.<sup>3</sup> The complete nucleotide sequence of the cryptic plasmid is now known<sup>4</sup> and a PCR protocol has been developed using primers derived from the integrated regions of the *cpxB* gene to identify the organism in clinical specimens, especially endocervical swabs.<sup>5,6</sup>

(ii) PPNG plasmids: High-level resistance to penicillin, observed among many strains of the *N. gonorrhoeae* has been found to be mediated by plasmids. Strains of the organism showing high-level resistance to penicillin are found to produce Penicillinase enzymes. These strains are conventionally called Penicillinase-producing *N. gonorrhoeae* (PPNG). Therefore, almost all PPNG strains are believed to harbour plasmids that confer genes for expressing beta-lactamase enzyme. The enzyme, as has been characterized among PPNG strains, is a TEM-1 type  $\beta$ -lactamase, which is carried on several related plasmids infecting few other species of bacteria.

The PPNG strains usually contain either of the plasmids,

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variously estimated as 3.2 to 3.4 MDa, or 4.4 to 4.7 MDa.<sup>7-9</sup> The 3.2 MDa-plasmid is designated as "Africa" type and the 4.4 MDa-plasmid as "Asia" type plasmid. Including these two, the Penicillinase production in isolates of *N. gonorrhoeae* have been found mediated by a family of six related  $\beta$ -lactamase-producing plasmids, which have been distinguished on their size, as determined by agarose gel electrophoresis.<sup>10-12</sup>

The PPNG plasmids have been named by their first geographical isolation source as Asia (7426 bp/ 4.4-4.7 MDa), Africa (5599 bp/ 3.2-3.4 MDa), Toronto (5154 bp/ 3.05 MDa), Rio (5154 bp/ 2.9 MDa), Nimes (6798 bp/ 4.0 MDa), and New Zealand (9309 bp/ 6.0 MDa) types.<sup>11-14</sup> Only the Asia, Africa and Toronto type plasmids have been associated with epidemic outbreaks while others have been isolated sporadically.<sup>10</sup>

A PCR assay was developed after aligning primary DNA sequences from Genebank and using manual alignments coupled with the Primer Designer Computer program (Scientific and Education Software) for the Asia, Africa and Toronto  $\beta$ -lactamase-producing plasmids (Genebank accession numbers U20374, U20375, and U20419, respectively). DNA sequences for the developed primers were: JDA (5'-TACTCAATCGGTAATTGGCTTC-3') and JDB (5'-CCATATCACCGTCGGTACTG-3').<sup>13</sup> Based on the DNA sequence of the gonococcal  $\beta$ -lactamase-producing plasmids, the predicted sizes of the amplicons for strains containing Asia, Africa or Toronto/ Rio-type plasmids were 4.9, 3.1 and 2.6 kb, respectively.<sup>13</sup>

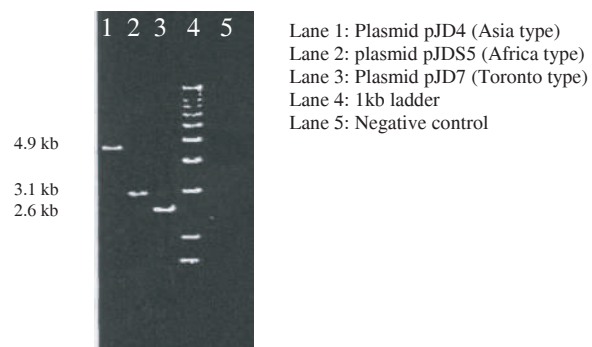


Figure 1: Agarose gel electrophoresis of the amplicons from control PPNG strains (reproduced with modification from Dillon *et al*, 1999<sup>13</sup>)

Based on restriction endonuclease analysis, the Asia type is supposed to be the ancestral plasmid from which other deletion or insertion derivatives have been generated.<sup>10,14</sup> The Africa plasmid type has been found to carry a 2.1 kb deletion in comparison with the Asia type plasmid. The Toronto and the Rio type plasmids are two other deletion derivatives being identical in size (5514 bp).<sup>15</sup> Due to similarity in size of these two plasmids, they are not differentiated in most epidemiological surveys and all plasmids between 2.9-3.05 MDa have been reported by some authors as Toronto type<sup>16-18</sup> and while as Rio plasmids by some others.<sup>19</sup> The insertion derivatives, on the other hand, are the Nimes (from Africa) and the New Zealand (from Asia) plasmids.<sup>10-12, 20</sup>

Table I: Worldwide reports of different types of PPNG plasmids

Reporting year	Country	No of PPNG	Type of PPNG	Rate	Authors
1982	Far East	27	Africa	00	Ng <i>et al</i> <sup>9</sup>
			Asia	100%	
1984	Zurich, Switzerland	30	Africa	03.3%	Eichmann & Pifferetti <sup>21</sup>
			Asia	96.7%	
1986	Rwanda	20	Africa	70.0%	Bogaerts <i>et al</i> <sup>22</sup>
			Asia	25.0%	
			Both	05.0%	
1987	Jamaica	20	Africa	70.0%	Dillon <i>et al</i> <sup>23</sup>
			Asia	30.0%	
1988	Munich, Germany	23	Africa	70.0%	Abeck <i>et al</i> <sup>24</sup>
			Asia	30.0%	
1991	London, UK	649	Africa	66.1%	Ison & Easmon <sup>25</sup>
			Asia	33.9%	
1992	Taiwan	-	Africa	00	Chu <i>et al</i> <sup>18</sup>
			Asia	82-95%	
			Toronto	5-18%	
1997	Bangkok, Thailand	26	Africa	96.2%	Knapp <i>et al</i> <sup>26</sup>
			Asia	03.8%	
1998	Bandung, Indonesia	26	Africa	00	Djajakusumah <i>et al</i> <sup>27</sup>
			Asia	100%	
1999	Bangladesh	22	Africa	100%	Bhuiyan <i>et al</i> <sup>28</sup>
			Asia	00	
2001	Caribbean countries	90	Africa	67.8%	Dillon <i>et al</i> <sup>29</sup>
			Asia	01.1%	
			Toronto	31.1%	

(iii) TRNG plasmids: High-level resistance to Tetracycline in *N. gonorrhoeae* (MIC $\geq$ 16.0  $\mu$ g/ml) has been found associated with plasmids. These strains of the organism, called TRNGs

(Tetracycline Resistant *N. gonorrhoeae*), have been shown to possess a 25.2 MDa plasmid, which has been thought to be derived by acquisition of a tet-M determinant from other species. The similar plasmids have also been found in *N. meningitidis*, *Kingella denitrificans* and *Eikenella corrodens* isolates.<sup>30</sup> These plasmids have an even wider host range in the laboratory.<sup>31</sup> The 25.2 MDa plasmid has been reported to share DNA homology with the previously described 24.5 MDa conjugative gonococcal plasmids.<sup>32,33</sup> A conjugative 25.2 MDa plasmid was also found carrying the tet-M gene to code for a large cytoplasmic protein that protects ribosomes from the action of Tetracycline.<sup>34, 35</sup>

*Two types of TRNG plasmids.* Restriction endonuclease analyses of conjugative plasmids carried by the TRNG isolates have indicated the existence of at least two plasmid types.<sup>36,37</sup> The restriction endonuclease map of the conjugative plasmid from a TRNG strain imported from the United States was found to differ from that of a Dutch TRNG isolate.<sup>36</sup> The 'American type' restriction pattern plasmid is an amplification product of 1600 bp and the 'Dutch type' restriction pattern has an amplification product of approximately 700 bp.<sup>36,37</sup> Studies on different TRNG plasmid types around the world has shown the Dutch type to be predominant in isolates from the Netherlands, Asia and South America, and a predominance of American type in isolates from the United Kingdom, and eastern and central Africa.<sup>37-39</sup>

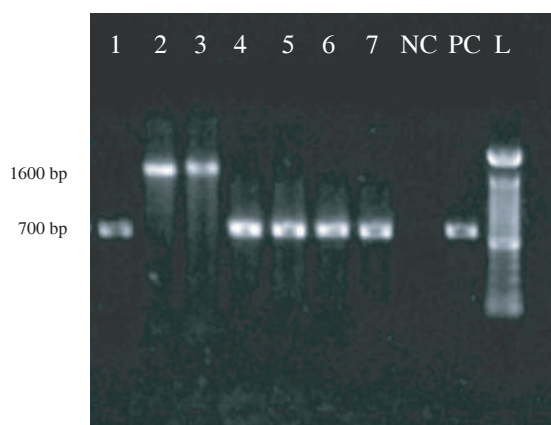


Figure 2: TRNG plasmids (reproduced from unpublished PhD thesis works of the author). Lanes 1, 4-7: Dutch type plasmids; Lanes 2,3: American type plasmids; NC- Negative control, PC- Positive control, L- 100 bp Ladder

Table II: Worldwide reports of different types of TRNG plasmids

Reporting year	Country	No of TRNG	Type of TRNG	Rate	Authors
1998	Bandung, Indonesia	470	Dutch	100%	Djajakusumah <i>et al</i> <sup>27</sup>
2001	Manaus, Brazil	62	Dutch	100%	Dillon <i>et al</i> <sup>40</sup>
2001	Cotonou, Benin	-	American	100%	van Dyck <i>et al</i> <sup>41</sup>

Penicillinase-producing *N. gonorrhoeae* (PPNG) is identified with a beta-lactamase (Penicillinase) test done by paper acidometric or iodometric methods or chromogenic cephalosporin test.<sup>42,43</sup> Whereas, non-PPNG strains of *N. gonorrhoeae* showing an MIC of greater than or equal to 16.0 µg/ml of Tetracycline are identified presumptively as having plasmid-mediated, high-level resistance to Tetracycline (TRNG). Isolates that produced beta-lactamase and have an MIC of greater than or equal to 16.0 µg/ml Tetracycline are classified as PPNG/TRNG.<sup>38</sup>

**Conjugation in Gonococci:**

Conjugation, as a process of gene recombination, where R-plasmids are transferred from resistant to sensitive genera of bacteria. It is very important in the onset and development of multiple resistance to the bacteria as well as it enables fast spreading of resistant genera in the human populations.

From 8% to 22% of unselected gonococci<sup>44,45</sup> and a higher proportion of Penicillinase producing (PPNG) gonococci were found to possess a 24 MDa conjugative plasmid that mobilizes transfer of plasmids (4.7 or 3.4 MDa PPNG plasmids) with high efficiency to other gonococci.<sup>46-49</sup> As many as 10% of cells carrying the 24 MDa plasmid may act as conjugal donors under suitable conditions,<sup>50</sup> suggesting that the gonococcal conjugal system normally is not repressed. Efficiencies of conjugation are reduced if donors or recipients contain heat-modifiable proteins related to colonial opacity.<sup>51</sup>

Attempts to visualize sex pili by electron microscopy were unsuccessful.<sup>44</sup> The 24 MDa conjugal plasmid apparently replicates only transiently in *Escherichia coli*.<sup>47</sup> A physical map of the plasmid has been prepared and restriction maps of 24 MDa plasmids isolated from different gonococcal strains are very similar, although they differ significantly in their efficiencies of mobilization of a PPNG plasmid.

It would be useful if gonococci possessed a conjugal system for chromosomal gene transfer. A report of chromosomal gene transfer by the 24 MDa plasmid was met with enthusiasm, but subsequent efforts to confirm this report have been unsuccessful.<sup>50,52,53</sup>

**Antibiotic resistance in Gonococci and the Plasmids:**

The development of antibiotic resistance in *Gonococcus* may involve either chromosomal or extrachromosomal (plasmid) mechanisms and for some antibiotics both may be implicated. Gonococcal acquisition of one of several types of plasmids might contain genetic elements coding for production of a TEM-type beta-lactamase giving rise to Penicillinase producing *N. gonorrhoeae* (PPNG). This capacitates *Gonococci* to disrupt Penicillins and can superimpose on any chromosomally induced changes in susceptibility.<sup>53,54</sup> Similarly, both chromosomal and plasmid-mediated genetic exchange have been identified in Tetracycline resistant *Gonococci*.<sup>54,55</sup>

Importantly, however, no plasmid-borne resistance to Cephalosporins or Quinolones has been detected in the pathogenic *Neisseriae*.<sup>57</sup>

**Geographical distribution of Plasmids in gonococci:**

*Gonococcal Plasmids in the Europe:*

Penicillin-resistant, beta-lactamase-producing gonococci were first reported in England in 1976.<sup>56-58</sup> Since then they have spread to other countries, and the number of infections with beta-lactamase-producing strains of *N. gonorrhoeae* was increasing.<sup>59</sup> Penicillin resistance in these organisms was found to mediate by an ampicillin-resistance-determinant carried on either a 5.3-kilobase (kb) (3.3 MDa) or a 7.4-kb (4.6 MDa) plasmid. Both plasmids carry about 40% of *TnA* 7.49,60 including the *bla* gene that encodes the TEM-1 beta-lactamase.<sup>49,56</sup> The 5.3-kb plasmids originated in  $\beta$ -lactamase-producing *N. gonorrhoeae* strains were isolated in the

Netherlands<sup>59,61</sup> and in Britain.<sup>62, 63</sup>

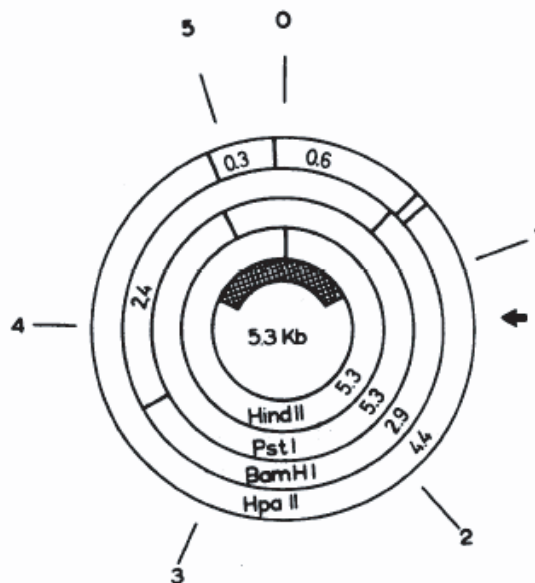


Figure 3: Restriction endonuclease cleavage map of the 5.3-kb plasmid PNG18. The hatched region represents the *TnA*-derived part of the plasmid. The arrow indicates the region where the 5.3-kb plasmid may have acquired an insertion element to become the 7.4-kb plasmid. (reproduced with permission from American Society for Medical Microbiologists)<sup>64</sup>

From 1981-1985, 2232 strains of *N. gonorrhoeae* were isolated and examined in the Venereal Diseases laboratory of Department for Dermatology, University Hospital of Zurich. Some 86 of the isolates were Penicillinase producing *N. gonorrhoeae* (PPNG) of which 42 (48.8%) had been found imported from South-East Asia. Six patients (6.9%) with PPNG strains had been identified become infected in Zurich. Plasmid patterns of all PPNG strains were determined. The 4.4 MDa-plasmid ("Asia" type plasmid) was found in 68 (79.1%) of 86 strains. The 3.2 MDa-plasmid ("Africa" type) in 8 (9.3%) of 86 and the 24.6 MDa-plasmid (transfer plasmid) in 56 PPNG strains were found respectively. The frequency of PPNG strains increased from 1.7% in 1981 to 6.7% in 1985.<sup>65</sup>

Twenty three Penicillinase producing strains of *N. gonorrhoeae* (PPNG) isolated in Munich from 1981 to 1986 were characterized in terms of their plasmid content. Sixteen strains (70%) harboured a 4.4 MDa plasmid (Asia type), with 13 of these strains also containing a 24.4 MDa plasmid. Seven strains (30%) contained a 3.2 MDa plasmid (Africa type), with only one of them containing a 24.4 MDa

plasmid.<sup>66</sup> On the other hand, 124 strains of non-Penicillinase-producing Gonococci isolated in Munich in 1986 were characterized in terms of their plasmid content. Seven (5.6%) of these strains contained the 24.4 MDa conjugative plasmid. Nine (8.6%) of the 105 strains, which harboured the cryptic plasmid also, found to contain the conjugative plasmid.<sup>67</sup>

A total of 1589 strains of *N. gonorrhoeae* isolated from patients attending St. Mary's Hospital, London were tested for plasmid profile, of which 649 were Penicillinase producing *N. gonorrhoeae* (PPNG). Among the PPNG strains, 429 carried the 4.4 MDa Penicillinase encoding plasmid and 220 carried the 3.2 MDa plasmid.<sup>24</sup>

The Penicillinase producing *N. gonorrhoeae* (PPNG) collected at St. Thomas' Hospital, UK from 1976-1990 were examined for plasmid profile. A total of 665 isolates of PPNG from patients attending the Department of Genitourinary Medicine at St. Thomas' Hospital were characterized. The 3.2 or 4.4 MDa plasmid, with or without the 24.5 MDa conjugal plasmid was seen in all isolates until 1989/90, when a 2.9 MDa beta-lactamase encoding plasmid and the 25.2 MDa plasmid-mediated Tetracycline resistance were also recognised.<sup>68</sup>

At the STD clinic of the Municipal Health Service in Amsterdam, The Netherlands, the annual number of PPNG strains remained relatively stable from 1983 until 1990. Among the total 1323 isolates of *N. gonorrhoeae*, PPNG were found in 415 (31.4%).<sup>69</sup> In another report from the department for infectious diseases epidemiology, National Institute of Public Health and the Environment, Bilthoven, Netherlands, in 1995, there was an overall high prevalence of PPNG infection (27%) and TRNG among PPNG infections (24%).<sup>70</sup>

#### *Gonococcal Plasmids in the Americas:*

Strains of *N. gonorrhoeae* carrying the Asia type of PPNG plasmids (reported size range 4.4-4.7 MDa) were first isolated in the USA in 1976<sup>59</sup> and named following their epidemiological origin in the Far East. Another report follows that identified Penicillin-resistant beta-lactamase producing Gonococci firstly in the USA in 1977.<sup>71</sup>

The 25.2 MDa Tet-M *Neisseria* plasmid showing high-level Tetracycline resistance was first isolated in the USA in 1983<sup>72</sup> and since then the TRNGs have become more widespread in the European countries including England<sup>33,73</sup> and the Netherlands.<sup>61</sup>

From 1988 to 1989, statistically significant increases ( $p$  less than 0.05) in the percentages of PPNGs occurred in seven clinics of USA. The PPNG rates remained approximately the same in the other 14 clinics in Atlanta, Birmingham, Boston, Long Beach, Philadelphia, San Antonio, and San Diego. In 1988, TRNG accounted for 4.0% isolates, in 1989, 4.9% isolates, and in both years, the percentage of infections with TRNG was the highest among clinics in the east and southeast of the US.<sup>74</sup>

An investigation conducted in the division of STD, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA to assess the role of Honolulu as a reservoir for PPNG strains by assessing the diversity and persistence of PPNG strains between 1982 and 1991. Of 432 *N. gonorrhoeae* isolates tested, 373 (86.4%) possessed a 4.4 MDa (Asia type) beta-lactamase plasmid; 39 (9.0%) possessed a 3.2 MDa (Africa type) plasmid; and 20 (4.6%) possessed a 3.05 MDa (Toronto type) beta-lactamase plasmid. Though all Toronto PPNG strains possessed a 24.5 MDa conjugative plasmid, these plasmids could not be transferred by conjugation. Although some apparent microepidemics of PPNG strains were identified, most strains were isolated sporadically.<sup>75</sup>

In a study observing antimicrobial susceptibility of *N. gonorrhoeae* isolated from three Caribbean countries showed that most (61/90, 67.8%) PPNG isolates carried Africa-type plasmids, with 928/90 (31.1%) having Toronto-type and a single isolate was found to carry an Asia-type plasmid. The tet-M determinant in the TRNG isolates was predominantly of the Dutch type (68/91, 74.7%).<sup>28</sup> Whereas, in another report from Manaus, Brazil, most of the isolates (85.2%) were resistant to Tetracycline, Penicillin or both with majority carrying plasmid-mediated resistance to Tetracycline (TRNG) and all of the TRNGs contained the Dutch type tet-M plasmid.<sup>39</sup> In another study conducted in Cuba found that majority of the isolates (63.4%) of *N. gonorrhoeae* exhibited plasmid-mediated resistance to Penicillin, Tetracycline or



both.<sup>76</sup>

In a retrospective determination of antimicrobial susceptibility of 91 isolates of *N. gonorrhoeae* from eleven centres in Cuba, 56% were PPNG and 64% were TRNG.<sup>77</sup>

#### *Gonococcal Plasmids in Africa:*

The *in vitro* sensitivity of 104 isolates of *N. gonorrhoeae* to six antimicrobial agents was tested in Rwanda and more than 50% of the isolates found to produce Penicillinase. Of the 20 PPNG isolates examined, 7 contained the 3.4 MDa R-plasmid, another 7 contained both the 3.4 MDa and 22.5 MDa plasmids, 5 contained the 4.3 MDa and 22.5 MDa plasmids, and 1 isolate harboured both the 3.4 and 4.3 R-plasmids, together with the 22.5 MDa plasmid.<sup>78</sup>

The analysis of the plasmid profiles of 459 non-PPNG strains from Jamaica suggests that strains have been imported to the island. These isolates were found to predominate in Canada and were increasingly reported from areas of the United States of America and Europe. Although most (70%, 14/20) PPNG strains harboured Africa type plasmids with or without transfer plasmids, 6 also carried Asia type plasmids (with the transfer plasmid).<sup>79</sup>

The prevalence of PPNG strains had been found steadily rising in Nigeria since 1979, and about 80% of the strains of Gonococci isolated in Ibadan were found to produce Penicillinase. Plasmid analysis shows that both "Asia" and "Africa" PPNG types were circulating in Nigeria.<sup>80</sup>

A total of 460 *N. gonorrhoeae* isolates from patients seen at three clinics in Dakar, Sénégal, during 1982-1986, had been investigated. Among Penicillin-resistant strains, the proportion of PPNG was fairly constant (range 35-55%). The study comprised 70 PPNG strains of which 19% (13/70) carried the 7.4 kb Asia type plasmid and 81% (57/70) the 5.3 kb Africa type plasmid.<sup>81</sup>

One hundred and fifty five strains of *N. gonorrhoeae* were regrown from 216 freeze-dried cultures originally isolated in Zimbabwe to see plasmid content and other epidemiological characteristics. Seventy five of the 100 isolates from STD clinic patients and 29 of the 55 isolates from hospital patients were PPNG. Two thirds of all PPNG strains contained the

24.5 MDa conjugative plasmid. The 3.2 MDa resistance plasmid, usually associated with PPNG strains originating in Africa, was present in only one third of the PPNG strains. The 2.6 MDa cryptic plasmid was present in all strains.<sup>82</sup>

Plasmid analysis in a study in Northern Tanzania showed that 65 strains (50%) were Penicillinase producers (PPNG), and the PPNG strains carried both the 3.2 MDa "Africa" type and the 4.4 MDa "Asia" plasmids, although the 3.2 MDa plasmid was more prevalent. Some 45 (35%) had high-level plasmid-mediated resistance (TRNG), all of which carried a 25.2 MDa plasmid.<sup>83</sup>

A cohort of 650 prostitutes (commercial sex workers) from Kinshasa, Zaire were followed at monthly intervals for sexually transmitted diseases as part of an HIV intervention project. *N. gonorrhoeae* were isolated and tested for antimicrobial susceptibility and plasmid profile. Among 1085 gonococcal isolates tested, 725 (67%) found to produce beta-lactamase (PPNG strains) and 323 (30%) showed plasmid-mediated resistance to Tetracycline (TRNG). Over time, the prevalence of PPNG varied between 60% and 73%, while the rate of TRNG increased from 11% to 45%.<sup>84</sup>

During various periods between 1988 to 1993, a total of 2,288 gonococcal isolates were obtained from three sites in West and Central Africa to determine antimicrobial susceptibility pattern and plasmid-mediated resistance to Penicillin. The prevalence of PPNG increased significantly over time from 44% to 57% in Kingali and remained stable at a high level in Abidjan (73%) and in Kinshasa (67%).<sup>85</sup>

In South Africa, 321 gonococcal strains were isolated from urethral exudates of adult men presenting with acute urethritis at the Sexually Transmitted Diseases Clinic at King Edward VIII Hospital in Durban during 1990-1993 to examine the *in vitro* susceptibility pattern and plasmid profile of PPNG strains. Some 17.8% of the strains were PPNG in the observation. During 1990-1993, the PPNG prevalence increased from 16.4% to 19%. All PPNG strains had the 2.6 MDa cryptic plasmid and 87.7% also had the 24.5 MDa conjugative plasmid. The investigators also observed that high prevalence of the conjugative plasmid in the PPNG strains may account for the increased prevalence of these strains. The predominant beta-lactamase plasmid type was the

4.4 MDa Asian plasmid (77.2%). The most common plasmid profile of the PPNG strains contained the 2.6 MDa cryptic, 24.5 MDa conjugative, and 4.4 MDa Asian plasmids (68.4%).<sup>86</sup>

One hundred and three strains of *N. gonorrhoeae* isolated from a periurban STD clinic in the Gambia were studied for antimicrobial susceptibility, plasmid profile, and serogroup using standard procedures. Seventy-nine (77%) were Penicillinase producers (PPNG) and fully resistant to Penicillin (MIC  $\geq$  8 mg/l) and 87 (84%) showed high-level plasmid-mediated resistance to Tetracycline (TRNG) (MICs  $>$  10 mg/l). This was the first report of TRNG in The Gambia. All PPNG and TRNG strains carried the 3.2 MDa and 25.2 MDa plasmids, respectively. All isolates carried the 2.6 MDa cryptic plasmid and 9 (3 PPNG and 6 non-PPNG) carried the 24.5 MDa conjugative plasmid.<sup>87</sup>

In another study in Kingali, Rwanda during 1985-1993, the investigators observed that the prevalence of PPNG remained stable at a rate of 39% during 1985-91 and increased to 61% in 1992-93. The steep increase of PPNG were the most striking facts comparable with findings from other African countries.<sup>88</sup>

Another study at Muhimbili Hospital, Dar es Salaam, Tanzania included 199 strains of *N. gonorrhoeae* isolated between 1993 and 1995, and identified a high rate (128/199, 64%) of PPNG strains.<sup>89</sup> Another very high rates of PPNG was observed in Kingali, Rwanda and the investigators reported that 35% PPNG in 1986 was increased significantly to 70.5% in 2000.<sup>90</sup>

Antimicrobial susceptibility pattern of *N. gonorrhoeae* isolates was obtained from female sex workers in Cotonou, Bénin. All isolates with a MIC of Tetracycline of  $>$ 8 mg/l carried the 'American type' tet-M plasmid; 94% and 6% of Penicillinase-producing isolates possessed a 3.2 MDa and a 4.4 MDa beta-lactamase plasmid, respectively.<sup>41</sup>

#### *Gonococcal Plasmids in Asian countries:*

The first report of gonococcal plasmids from Singapore found that the incidence of PPNG infections increased alarmingly from 3 cases in 1976 to 1,792 cases (19.2% of total gonococcal infections) in 1979. This increase was paralleled

by a decrease in the number of non-PPNG cases from 8,036 in 1978 to 7,540 in 1979. Female prostitutes contributed 72.7% of PPNG infections in 1979.<sup>91</sup>

A 4.4 MDa plasmid, associated with beta-lactamase production, was found in all 27 PPNG isolates examined in the Far East in 1979 and 1980; 93% of PPNG and 22% of non-PPNG isolates found to contain a 24 MDa plasmid associated with transfer of the 4.4 MDa plasmid.<sup>92</sup>

One hundred strains of *N. gonorrhoeae* (including 30 PPNG strains) originating from Korea were characterized to see plasmid content. Eighty per cent of the isolates possessed the conjugative 24.5 MDa plasmid. A novel 7.8 MDa plasmid was present in four isolates (one PPNG and three non-PPNG strains).<sup>93</sup>

Since first diagnosis of PPNG in Singapore, the prevalence of PPNG has increased progressively, and maintained at between 30% to 35% of all gonococcal infections in spite of a decrease in the overall incidence of gonorrhoea in Singapore during the late Nineties. The PPNG infections have been recorded with all forms of complicated gonorrhoea including pelvic inflammatory disease, ophthalmia and disseminated gonococcal infection. The medical cost of treatment of gonorrhoea has increased by 100 to 200% as a result of the emergence of PPNG.<sup>94</sup> In another study in Singapore, 60 randomly collected *N. gonorrhoeae* isolates from 41 symptomatic, untreated males and 19 female prostitutes were investigated to characterize *N. gonorrhoeae* isolates by plasmid analysis. Analysis of the 25 PPNG strains showed that 16 (64%) of them carried the 4.4 MDa (Asian type) plasmid and 9 (36%) strains harboured the 4.4 MDa plasmid in conjunction with the 24.5 MDa transfer plasmid. The cryptic plasmid of 2.6 MDa was present in 27 of the 35 non-PPNG strains. Five of the non-PPNG strains harbouring the cryptic plasmid also contained the 24.5 MDa transfer plasmid. Others also observed a plasmid combination of 2.6 MDa + 7.8 MDa + 24.5 MDa detected in three non-PPNG strains.<sup>95</sup>

The PPNG strains first appeared in Taiwan in late 1976, and the first six of the PPNG isolates were from United States military servicemen who had relocated from Southeast Asia. The percentage of PPNG strains rose to 37.82% in 1982, and

remained high (50%-62%) since 1983. Two kinds of R plasmids were isolated in the PPNG strains: the 4.4 MDa Asia type (82%-95%) and the 3.05 MDa Toronto type (5%-18%). Evidences suggested that the Asia type R plasmid was imported into Taiwan in 1976, while the Toronto-type R plasmid may have first emerged in Taiwan in 1983.<sup>96</sup>

During July-October, 1994 in the Philippines, *N. gonorrhoeae* were isolated from 92 female sex workers in Manila and Cebu City to characterize the gonococcal strains by plasmid content and the PPNG comprised 70.7% of the isolates. Strains with the 3.2 MDa beta-lactamase plasmid were more common in Cebu City than in Manila (57.8% vs. 28.6%; p = 0.02). They had significantly lower MICs to Penicillin, Tetracycline, Ceftriaxone, Ciprofloxacin, and Erythromycin than did strains with 3.05-, 3.9-, or 4.4 MDa plasmid (p 0.01). One PPNG strain had a previously undescribed 3.9 MDa beta-lactamase plasmid. It also had a 24.5 MDa conjugative plasmid. Tetracycline-resistant *N. gonorrhoeae* (TRNG) comprised 6.5% of the isolates.<sup>97</sup>

Some 50 consecutive isolates of *N. gonorrhoeae* were studied in India during 1995 to 1996, of which 8% were PPNG while 28% were highly resistant to Tetracycline (TRNG). All PPNG harboured the 4.4 MDa β-lactamase plasmid (Asia type) along with the 25.2 MDa Tetracycline resistance plasmid.<sup>98</sup>

Typing of the Tet-M plasmid in Tetracycline-resistant isolates (TRNG) was performed in Bandung, Indonesia using a polymerase chain reaction (PCR) technique and plasmid profiles of PPNG were determined. All PPNG possessed the 4.4 MDa beta-lactamase plasmid (Asia type) and all TRNG showed a PCR fragment characteristic of the 'Dutch' type Tet-M plasmid. Of the 50 gonococci isolates tested, all were resistant to Tetracycline; 47 (94%) were TRNG, 26 (52%) were PPNG.<sup>26</sup>

A study concerning the prevalence of gonococcal infection among commercial sex workers (CSWs) was conducted in Bangladesh. The plasmid profile of the isolates was also analyzed. Among the resistant isolates, 23.4% were PPNG and 17.5% were Tetracycline-resistant *N. gonorrhoeae*. All PPNG isolates contained a 3.2 MDa African type of plasmid, and a 24.2 MDa conjugative plasmid was present in 34.1% of

the isolates.<sup>28</sup>

Another investigation in India was carried out to determine the prevalence of PPNG, and plasmid profile among women attending tertiary level health centers and sexually transmitted disease (STD) clinics in Mumbai (formerly Bombay). In plasmid pattern, 10 of the PPNG isolates possessed the 4.4 MDa plasmid (Asia type), while 4 had the 3.2 MDa plasmid (Africa type). The 4.4 MDa plasmid was the most prevalent among the PPNG isolates.<sup>99</sup>

Table III: Countries worldwide reporting high-rates of PPNG strains

Reporting year	Continent/ City/ Country	No. of PPNG	Percent of PPNG	Authors
<b>Asia</b>				
1982	Far East	27/36	75.0%	Ng <i>et al</i> <sup>9</sup>
1992	Taiwan	-	50-62% (1983)	Chu <i>et al</i> <sup>18</sup>
1997	The Philippines	65/92	70.7%	Knapp <i>et al</i> <sup>97</sup>
1999	Mumbai, India	15/33	46.7%	Divekar <i>et al</i> <sup>99</sup>
2003	Java, Indonesia	111/180	61.7%	Ieven <i>et al</i> <sup>100</sup>
2006	Bali, Indonesia	117/147	79.1%	Donegan <i>et al</i> <sup>101</sup>
<b>Africa</b>				
1988	Harare, Zimbabwe	104/155	67.0%	Mason & Gwanzura <sup>82</sup>
1995	Northern Tanzania	65/130	50.0%	West <i>et al</i> <sup>83</sup>
1995	Zaire	725/1085	67.0%	Van Dyck <i>et al</i> <sup>84</sup>
1997	Kingali	542/952	57%	Van Dyck <i>et al</i> <sup>85</sup>
	Abidjan	183/251	73.0%	
	Kinshasa	727/1085	67.0%	
1997	Gambia	79/103	77.0%	Adegbola <i>et al</i> <sup>87</sup>
1998	Kingali, Rwanda	-	61.0% (92-93)	Bogaerts <i>et al</i> <sup>88</sup>
1999	Dar es Salaam, Tanzania	128/199	64.0%	Mbwana <i>et al</i> <sup>89</sup>
2001	Kingali, Rwanda	-	70.5%	Van Dyck <i>et al</i> <sup>90</sup>
<b>Europe</b>				
1994	The Netherlands	415/1323	31.4%	Prins <i>et al</i> <sup>69</sup>
1997	The Netherlands	-	27.0%	Van de Laar <i>et al</i> <sup>70</sup>
<b>South America</b>				
2003	Cuba	51/91	56.0%	Sosa <i>et al</i> <sup>77</sup>

A study was undertaken recently in Bandung and Jakarta,



Indonesia, isolated *N. gonorrhoeae* from female commercial sex workers and characterized the Tet-M plasmid among the Tetracycline-resistant strains by PCR. Prevalence of Penicillin and Tetracycline resistance was extremely high: 60.0% of the isolates from Bandung and 70.9% of the isolates from Jakarta were resistant to Penicillin. Of these, 60.0% and 62.1%, respectively, were PPNG. All Tetracycline-resistant isolates from Bandung and 97.8% from Jakarta carried a PCR fragment characteristic of the "Dutch" type Tet-M plasmid.<sup>100</sup> Another very recent report from Bali, Indonesia found very high rates of PPNG. Characterizing 147 strains of *N. gonorrhoeae*, the investigators reported that all were highly resistant to Tetracycline (MIC >16 µg/ml) and 117 (79.1%) of them were beta-lactamase positive (PPNGs).<sup>101</sup>

A very different picture was observed in a study in Japan, and the prevalence of PPNG strains significantly decreased from 7.9% in 1993-1994 to 2.0% in 1997-1998. No TRNG or PPNG-TRNG isolate was detected during the study period of 1993 through 1998.<sup>102</sup> A similar decreasing pattern of plasmid-mediated resistance among *N. gonorrhoeae* isolates was recorded by the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP) in their annual Report in 2006. Some 9.5% of isolates demonstrated Penicillin resistance in 2006 (MIC =1mg/l or β-lactamase positive), down from 17.9% in 2005 (p=0.01). A decrease was observed in both the prevalence of PPNG or PP/TRNG in heterosexual males from 7.5% in 2005 to 4.8% in 2006, and amongst women from 4.6% in 2005 to 2.6% in 2006. However, an increase in the prevalence amongst male individuals having sex with male (MSM) was observed, rising from 0.8% in 2005 to 2.2% in 2006.<sup>103</sup>

The GRASP report also found a little increase in Plasmid-mediated Tetracycline resistance (TRNG or PP/TRNG) and observed in 7.7% of isolates in 2006 compared to 7.1% in 2005. There were increases in the prevalence of TRNG or PP/TRNG amongst both heterosexual men 9.1% in 2006 (6.5% in 2005) and women 6.1% in 2006 (5.4% in 2005). Whilst the prevalence decreased in MSM from 8.8% in 2005 to 7.0% in 2006, although none of these changes were statistically significant. The prevalence of Tet-R decreased amongst heterosexual males from 18.3% in 2005 to 14.5% in

2006 and in MSM from 45.0% in 2005 to 42.9% in 2006. However, the prevalence of Tet-R amongst women decreased significantly from 14.6% in 2005 to 7.5% in 2006 (p=0.05).<sup>103</sup>

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