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Characteristics of gonococci isolated from men with urethritis in Dubai

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SUMMARY

Neisseria gonorrhoeae were collected from men attending out-patient clinics in Dubai. The susceptibility to a range of therapeutic antibiotics and their auxotype and serotype was determined. The plasmid content of all penicillinase-producing strains was also analysed. Thirty-six strains of *N. gonorrhoeae* were isolated from specimens collected from 79 patients over a 24-day period. Of the 36 isolates, 9 (25%) were penicillinase-producing *N. gonorrhoeae* (PPNG) and 15 (42%) were chromosomally resistant *N. gonorrhoeae* (CMRNG). CMRNG exhibited higher levels of resistance to cefuroxime, chloramphenicol, tetracycline and erythromycin than PPNG. All isolates were susceptible to ceftriaxone and spectinomycin. Three (8%) isolates showed reduced susceptibility (MIC, $\geqslant$ 0·25 mg/l) to ciprofloxacin. Six isolates of PPNG carried the 4·4 MD and three the 3·2 MD penicillinase encoding plasmid. The total gonococcal population was phenotypically diverse, with 12 serovars, 6 auxotypes and 21 A/S classes. Gonorrhoea was found to be a major cause of urethritis in Dubai and the strains exhibited high levels of resistance to penicillin.

INTRODUCTION

The incidence of gonorrhoea in Europe [1] and North America has been falling dramatically in the last decade. This has been primarily attributable to public health measures, effective antimicrobial therapy given early in the disease and changes in sexual practice since the advent of the Acquired Immunodeficiency Syndrome. In contrast the incidence of gonorrhoea continues to increase in the poorer countries of Africa and Asia due to a lack of appropriate facilities and finance to provide effective control measures [2]. In certain parts of the developing world effective antibiotic therapy is either unavailable or too expensive. Inappropriate antibiotics are, therefore, often used with the potential to encourage the development of resistance.

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In countries of the Middle East, such as the United Arab Emirates (UAE), there are no established procedures for screening and treating sexually transmitted diseases which can lead to many of the problems encountered in African and Asian countries. As a consequence the prevalence of gonorrhoea in the Middle East is largely unknown and there is limited information on levels or types of antibiotic resistance [3–5]. Unlike many African and Asian countries, however, UAE and neighbouring countries have the facilities and antibiotics to control the spread of sexually transmitted infections, if used appropriately.

Dubai is the second largest of the seven emirates that together make up the United Arab Emirates (UAE). The member states all share a common religion and culture. UAE has developed rapidly over the last two decades following the discovery of oil. This has changed lifestyles and has attracted migrant

workers in search of higher salaries from diverse geographical areas.

The aim of this study was to collect preliminary data on the susceptibility to therapeutic agents of isolates of *N. gonorrhoeae* from male patients in Dubai and to use phenotypic typing methods to give an indication of the diversity of the gonococcal population.

MATERIALS AND METHODS

Patient population

Urethral swabs and/or urine were collected from 79 consecutive men with a urethral discharge or dysuria attending the dermatology or urology clinic at four hospitals (Al-Kuwait, Rashid, New Dubai, Al-Irani) and three private clinics in Dubai during December 1992 and January 1993. Urethral swabs were collected when possible. However, there were occasions when only urine samples were available. The urine was centrifuged and the deposit used for isolation of *N. gonorrhoeae*.

Isolation of *N. gonorrhoeae*

Specimens were inoculated onto GC agar base (Difco Laboratories) supplemented with 1% IsoVitaleX and made selective by the addition of vancomycin (3 mg/l), colistin (100 units/ml), trimethoprim (5 mg/l) and amphotericin B (1·5 mg/l) and incubated at 36 °C in 6% carbon dioxide for 48 h. Colonies that were oxidase positive Gram-negative cocci were subcultured onto non-selective medium and the growth harvested into 15% glycerol broth for storage at −70 °C or −40 °C until transported to London.

On arrival in London, all strains were retrieved by subculturing on non-selective GC agar supplemented with 1% IsoVitaleX. All oxidase positive, Gram-negative cocci were confirmed as *N. gonorrhoeae* using immunofluorescence (Syva GC Microtrak).

Antibiotic susceptibility testing

The breakpoint agar dilution was used to allow categorization of strains into susceptible, reduced susceptibility (or intermediate resistance) and resistant [6]. The concentrations used were penicillin (0·06, 0·5 mg/l), cefuroxime (0·06, 0·5 mg/l), erythromycin (0·25, 2·0 mg/l), chloramphenicol (0·25, 2·0 mg/l), tetracycline (0·25, 4·0 mg/l), spectinomycin (32 mg/l), ciprofloxacin (0·03, 0·12 mg/l) and ceftriaxone (0·03, 0·12 mg/l). The medium was Diagnostic Sensitivity Test (DST) Agar (Unipath Ltd) supplemented with 1% IsoVitaleX and 5% lysed horse blood. The inoculum was 10^4 colony forming units (cfu) and the presence and absence of growth was scored after 48 h incubation at 36 °C in 6% carbon dioxide. The World Health Organization control strains A–E together with a strain exhibiting reduced susceptibility to ciprofloxacin were used as controls. The full minimum inhibitory concentration (MIC) was also determined for strains exhibiting resistance to penicillin and ciprofloxacin.

High-level plasmid-mediated resistance to tetracycline was screened for using GC agar base supplemented with 1% IsoVitaleX and 10 mg/l tetracycline. Growth after 24 h incubation at 36 °C in 6% carbon dioxide is predictive of the presence of the *tetM* determinant [7]. Plasmid-mediated penicillinase production was identified using the chromogenic cephalosporin (Nitrocefin, Unipath Ltd).

Plasmid analysis

PPNG were inoculated onto GC agar base supplemented with 1% IsoVitaleX and 1 mg/l of penicillin and incubated overnight at 36 °C in 6% carbon dioxide. Plasmids were extracted using a modification of the method described by Birnboim and Doly [8].

Auxotyping and serotyping

The chemically defined medium of Copley and Egglestone [9] was used to determine the requirement of all strains for proline, arginine, hypoxanthine, uracil, methionine and histidine. Arginine requiring strains were also tested for their ability to use ornithine as an alternative substrate. Each medium was inoculated with 10^4 cfu and scored for macrocolonies after 24 h incubation at 36 °C in 6% carbon dioxide.

Serological classification of all strains was performed using the method and nomenclature of Knapp and colleagues [10]. The GS (Genetic Systems) panel of 12 monoclonal antibodies raised to specific epitopes on the two types of the major porin, PIA and PIB, was used. A boiled suspension of each strain was mixed with each of the monoclonal antibodies linked to

staphylococcal Protein A (Calbiochem) for 2 min as described previously [10]. The reaction pattern designated the serovar.

RESULTS

A total of 36 isolates of *N. gonorrhoeae* were isolated from specimens collected from 79 men attending four hospitals, Al-Kuwait (3 isolates from 8 specimens), Rashid (7 isolates from 14 specimens), New Dubai (11 isolates from 14 specimens), Al-Irani (12 isolates from 38 specimens) and three private clinics (3 isolates from 5 specimens) in Dubai. Of the 36 isolates, 24 were isolated from urethral swabs, 9 from urine and 3 from both. When *N. gonorrhoeae* was isolated from both the urethral swab and urine on the same patient, only the isolate from the urethral swab was stored for further testing. All strains were successfully retrieved in London and were available for testing. The mean age of the men infected with *N. gonorrhoeae* was 27·8 years (range 16–50). Only ten of the men originated from the United Arab Emirates. The remaining patients were from other countries of the Middle East; Iran (6), Egypt (4), Sudan (1) and Syria (1), or from India (3), Pakistan (8) or Bangladesh (1). Two patients were of unknown origin.

Antibiotic susceptibility

Nine (25%) of the 36 gonococcal strains were penicillinase producing *N. gonorrhoeae* (PPNG). None exhibited high-level plasmid mediated resistance to tetracycline. Of the 27 strains of non-PPNG, 15 (56%) exhibited chromosomal resistance to penicillin (MIC, $\geqslant$ 1 mg/l), with only one strain exhibiting full susceptibility (MIC, $\leqslant$ 0·06 mg/l) (Table 1). The 15 strains of CMRNG had MICs to penicillin of 1–2 mg/l. There were also high levels of chromosomal resistance among non-PPNG to cefuroxime, chloramphenicol and tetracycline and to a lesser degree to erythromycin. In comparison, isolates of PPNG were more susceptible to these antibiotics (Table 1).

All isolates were susceptible to ceftriaxone (MIC, $\leqslant$ 0·03 mg/l) and spectinomycin (MIC, $\leqslant$ 32·0 mg/l). The majority of isolates (32 of 36, 89%) were fully susceptible to ciprofloxacin (MIC, $\leqslant$ 0·03 mg/l). Four isolates (3 isolates of non-PPNG and 1 PPNG) had a MIC of $\geqslant$ 0·06 mg/l. Three isolates exhibited a MIC of 0·25–0·5 mg/l which could be considered to be potentially resistant [6].

Table 1. Susceptibility of *27 nonPPNG and 9 PPNG strains isolated in Dubai*

	Number of strains (%)	
Antibiotic (mg/l)	Non-PPNG	PPNG
Penicillin		
$\leqslant$ 0·06	1 (4)	0
0·12–0·5	11 (41)	0
$\geqslant$ 1·0	15 (55)	9 (100)
Cefuroxime		
$\leqslant$ 0·06	2 (7)	2 (22)
0·12–0·5	15 (56)	7 (78)
$\geqslant$ 1·0	10 (37)	0
Erythromycin		
$\leqslant$ 0·25	1 (4)	1 (11)
0·5–2·0	23 (85)	7 (78)
$\geqslant$ 4·0	3 (11)	1 (11)
Chloramphenicol		
$\leqslant$ 0·25	0	0
0·5–2·0	6 (22)	3 (33)
$\geqslant$ 4·0	21 (78)	6 (67)
Tetracycline		
$\geqslant$ 0·25	0	0
0·5–4·0	14 (52)	6 (67)
$\geqslant$ 8·0	13 (48)	3 (33)
Spectinomycin		
$\leqslant$ 32·0	27 (100)	9 (100)
Ciprofloxacin		
$\leqslant$ 0·03	24 (89)	8 (86)
0·06–0·12	1 (4)	0
$\geqslant$ 0·25	2 (7)	1 (11)
Ceftriaxone		
$\leqslant$ 0·03	27 (100)	9 (100)
0·06–0·12	0	0
$\geqslant$ 0·25	0	0

Plasmid profiles

Six of the nine isolates of PPNG carried the 4·4 megadalton (MD) penicillinase encoding plasmid and two of these also carried the 24·5 MD conjugative plasmid. The remaining three isolates carried the 3·2 MD penicillinase plasmid, of which one also carried the 24·5 MD plasmid. All isolates carried the 2·6 MD cryptic plasmid.

Auxotyping and serotyping

In the total population, 12 serovars, 6 auxotypes and 21 auxotype/serovar (A/S) classes were found. All 21 A/S classes were found among non-PPNG, of which 7 were also found among PPNG (Table 2). The most common serovars were IB-1, IB-3 and IA-1/2 accounting for 64% of the total population. Non-

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Table 2. *Distribution of auxotypes and serovars (non-PPNG, 27 isolates and PPNG, 8 isolates. One PPNG isolate was not available for testing)*

Serovars	No. (%)	Auxotype* Total number [No. of PPNG]					
		NR	Pro	Meth	PAM	AM	PAOM
IA-1/2	4 (11)	1 [1]	3	.	.	.	.
IA-6	2 (6)	.	1	.	1	.	.
IA-16	1 (3)	.	1 [1]	.	.	.	.
IB-1	13 (36)	5	2	.	4	1	1
IB-3	6 (17)	4 [2]	1	1	.	.	.
IB-3/6D9	2 (6)	1 [1]	.	.	1	.	.
IB-4	1 (3)	1 [1]	.	.	.	.	.
IB-5	1 (3)	.	1 [1]	.	.	.	.
IB-8	2 (6)	2	.	.	.	.	.
IB-18	1 (3)	.	1 [1]	.	.	.	.
IB-22	1 (3)	1	.	.	.	.	.
IB-23/6D9	1 (3)	.	1	.	.	.	.
Total	35	15	11	1	6	1	1

* NR, non-requiring; Pro, proline requiring; Meth, methionine requiring; PAM, proline, arginine and methionine requiring; AM, arginine and methionine requiring; PAOM, proline, arginine, ornithine and methione requiring.

requiring and proline requiring were the predominant auxotypes accounting for 74% (26 of 35 isolates). Of the 21 A/S classes, NR/IB-1 was the most common with only 5 isolates (14%).

DISCUSSION

We have examined a small collection of random isolates of *N. gonorrhoeae* from men attending clinics in Dubai. The gonococcal population was found to be heterogeneous and to exhibit high levels of resistance to penicillin.

There is only limited information available on gonorrhoea in Dubai or UAE. Al-Rustamani studied gonorrhoea at a single hospital in Dubai between 1990 and 1992 [3]. The isolation rate of *N. gonorrhoeae* was highest amongst men that were not from UAE rather than among local men [3]. Previously, in 1981, Farid and colleagues [11] studied patients attending an out-patients clinic in Abudhabi over a 2·5 year period and showed that gonococcal urethritis was the most common disease encountered, followed by syphilis and trichomoniasis.

In this study the isolation of gonorrhoea among men with urethritis was 46% (36 isolates from 79 specimens). This shows that in symptomatic men who have sought treatment, *N. gonorrhoeae* is a major cause of infection. The isolation rate was highest in migrant men and this may reflect the high percentage of migrants in the UAE population. The migrant population is often comprised of young people and is likely to travel particularly to their country of origin and hence increase the chance of imported infection. Most of the migrant men are in UAE without their families and this may also lead them to use sex workers or indulge in casual sexual intercourse.

It is not possible from this study to determine the prevalence of gonorrhoea because only patients with symptoms have been screened. In addition, with no designated centres for STD diagnosis, it is likely that many patients are treated empirically without investigation. The lack of any specialized clinics for STDs means that patients who are at risk but asymptomatic will probably not be screened for STDs. This is particularly important for women who are reluctant to attend clinics because of cultural and religious pressures and who may be unaware of harbouring an asymptomatic infection.

In addition to the lack of specialized clinics there is also no policy for first line therapy for gonorrhoea. Of the 36 isolates in this study, 9 (25%) were penicillinase-producing and 15 (42%) exhibited chromosomal resistance to penicillin. In total, 24 (67%) isolates showed resistance to penicillin that could result in therapeutic failure. Penicillin resistance has also been reported from Riyadh, Saudia Arabia (12% PPNG, 13% CMRNG among 83 isolates) [4], from Tripoli, Libya (35·7% PPNG, 9·5% CMRNG among 42 isolates) [5], and from Bahrain (45% CMRNG among 91 non-PPNG) [12]. The gonococcal isolates

tested in this study did, however, show susceptibility to alternative therapies such as ceftriaxone and spectinomycin. Four isolates showed reduced susceptibility to ciprofloxacin of which three had a MIC $\geqslant$ 0·25 mg/l. The relationship between dosage, MIC and therapeutic failure for ciprofloxacin is unclear but isolates with MICs of 0·25 mg/l have been associated with therapeutic failures using a 250 mg single oral dose [13]. Our experience at St Mary's Hospital, London is that such isolates are therapeutically sensitive to a 500 mg single dose. There is, however, evidence of increasing levels resistance to ciprofloxacin in *N. gonorrhoeae* (14, 15) and the emergence of strains exhibiting high-levels of resistance with MICs to ciprofloxacin of 16 mg/l (16, Dr J. Tapsall, Sydney, Australia, personal communication). The results in this study indicate that any policy for treatment should not include penicillin but that the newer single-dose treatments for gonorrhoea such as ceftriaxone, ciprofloxacin and spectinomycin should be successful. Antibiotics such as erythromycin and tetracycline which are less expensive alternatives should be discouraged because of the high levels of chromosomal resistance found in this population.

The total gonococcal population was phenotypically diverse belonging to 21 A/S classes in contrast to other studies we have performed on isolates from Bahrain [12] and The Gambia [17] which were more homogeneous. The small number of PPNG isolates were also heterogeneous in that four different plasmid combinations (4·4 MD alone, 3·2 MD alone, 4·4 MD + 24·5 MD, 3·2 MD + 24·5 MD) were found and the isolates belonged to seven A/S classes. The 4·4 MD plasmid was first detected in an isolate from Asia and the 3·2 MD plasmid from isolates epidemiologically linked with Africa. However, these plasmids are now found in PPNG from most parts of the world. The diversity of the population suggests that the gonococci originate from a number of different sources including those outside UAE.

We have shown that gonorrhoea is a major cause of symptomatic urethritis in men in Dubai. This study does not establish the true prevalence of gonorrhoea in Dubai or address the problem of undetected gonorrhoea in women. Asymptomatic infection in women almost certainly acts as a reservoir of infection but may also have serious consequences for women's health. Treatment is available for those who wish to seek it but the lack of any defined antibiotic policy based on appropriate surveillance data will result in the use of antibiotics liable to fail therapeutically, such as penicillin or tetracycline. If control measures are to be effective in countries of the Middle East more information is required on the prevalence of gonorrhoea and surveillance of susceptibility to antibiotics needs to be instigated.

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