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SURVEILLANCE OF ANTIBIOTIC RESISTANCE IN INVASIVE ISOLATES OF NEISSERIA MENINGITIDIS IN AUSTRALIA 1994–1999

NATIONAL NEISSERIA NETWORK OF AUSTRALIA

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Summary

A total of 1434 strains of *Neisseria meningitidis* isolated from cases of invasive meningococcal disease (IMD) in Australia between 1994 and 1999 were examined by standard methods for susceptibility to antibiotics used for treatment and prophylaxis. The proportion of isolates fully susceptible to penicillin decreased from 45% in 1994 to 26% in 1999 ($P < 0.001$). All the other isolates were less sensitive to penicillin except for two meningococci with a penicillin MIC of 1 mg/l. The geometric mean penicillin MIC increased from 0.045 to 0.065 mg/l from 1994 to 1999. There was no significant difference in the geometric mean penicillin MICs of serogroup B and serogroup C meningococci. Penicillin susceptibility was significantly associated with a poorer outcome. Isolates from survivors of IMD had a higher geometric mean penicillin MIC (0.06 mg/l) than those from fatal cases (0.048 mg/l) ($P < 0.001$). This suggests that factors other than the decrease in susceptibility to penicillin observed were more relevant to outcome in IMD. All isolates were fully susceptible to ceftriaxone. Rifampicin resistance was infrequent (eight isolates in 6 years) and sporadic. A single isolate had decreased quinolone susceptibility. Despite the significant shift in susceptibility to penicillin recorded, this group of antibiotics remains a suitable treatment for IMD in Australia.

Key words: Neisseria meningitidis, meningococci, surveillance, antibiotics, penicillin, rifampicin, quinolone, ceftriaxone.

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INTRODUCTION

Antibiotics have an essential role in the management of invasive meningococcal disease (IMD) as both therapeutic and chemoprophylactic agents. In general, resistance to antibiotics in *Neisseria meningitidis* has been slow to evolve and as yet does not appear to pose a significant threat to disease outcome. However, there is a large number of overseas reports, some of long standing, of isolates of *N. meningitidis* from cases of IMD with decreased susceptibility to the penicillin group of antibiotics[1–5] and the proportion of isolates with this decreased susceptibility varies widely over time and in different countries. The altered penicillin susceptibility appears to be the result of acquisition by meningococci of genes coding for altered penicillin-binding proteins from commensal *Neisseria* species by a process of transformation.[6,7] A small number of meningococci have also been shown to be penicillinase producing.[8–11] High level resistance to chloramphenicol in meningococci, mediated by production of chloramphenicol acetyltransferase, has also been recently reported,[12] but this antibiotic is now rarely used for treatment of IMD in Australia. Resistance to the later generation cephalosporins has not been reported in meningococci.

The proportion of strains resistant to the sulphonamides, once used extensively for chemoprophylaxis of IMD, is such that their use for this purpose has been discontinued. There are also reports, both overseas and in Australia, of sporadic resistance to rifampicin which is currently the most widely used chemoprophylactic agent used for IMD. Both permeability and *rpoB* mutations play a role in rifampicin resistance in meningococci.[13–15] Ciprofloxacin is also a recommended chemoprophylactic agent in IMD, and a meningococcus with decreased quinolone susceptibility and GyrA changes has been described in a local isolate from blood culture.[16]

The National Neisseria Network (NNN) has monitored the antibiotic susceptibility of meningococci as part of its continuing surveillance of isolates from cases of IMD in Australia.[17] This report summarises and analyses NNN data generated over the 6 years from 1994 to 1999 on the antibiotic susceptibility of meningococci from cases of IMD to agents used in treatment and for chemoprophylaxis in Australia. Particular attention was paid to trends in penicillin susceptibility.

MATERIALS AND METHODS

The NNN is a long-term continuing collaborative programme of surveillance of invasive isolates of *N. meningitidis*.[17] It supplements data from clinical notification systems by determining the phenotype and genotype of invasive meningococci. Also included in NNN datasets were details of age, site of isolation and clinical outcome of cases of IMD (survived or died).

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TABLE 1 Trends in penicillin susceptibility of invasive strains of *Neisseria meningitidis* isolated in Australia, 1994–1999

Year	No.	% S	% LS	No. R	MIC range (mg/l)	Geo mean MIC (mg/l)
1994	167	45.5	54.5	0	0.016–0.25	0.045
1995	201	34.3	64.7	0	0.016–0.5	0.063
1996	246	27.2	72.7	1	0.016–1	0.057
1997	310	22.5	77.5	0	0.016–0.5	0.066
1998	228	18.8	81.2	0	0.016–0.5	0.066
1999	282	26.2	73.7	1	0.016–1	0.065

S, sensitive, MIC$\leqslant$0.03 mg/l; LS, less sensitive, MIC 0.06–0.5 mg/l; R, relatively resistant, MIC$\geqslant$1 mg/l. Geo mean, geometric mean MIC.

Antibiotic susceptibility data were generated in the laboratories of the NNN by the determination of MICs using standardised agar-plate dilution techniques, Isosensitest agar (Oxoid, Basingstoke, UK) with added 8% saponin-lysed horse blood as the test medium and an inoculum of 10^4 colony-forming units per spot.[18] Quantitative sensitivities to penicillin, ceftriaxone, rifampicin and ciprofloxacin were determined for meningococci from cases of IMD isolated in or referred to NNN laboratories. Isolates were categorised as sensitive to penicillin, ciprofloxacin and ceftriaxone when MICs derived by this method were $\leqslant$0.03 mg/l, as less sensitive in the range 0.06–0.5 mg/l and relatively resistant when MICs were $\geqslant$1 mg/l. Rifampicin resistance was defined as an MIC $\geqslant$1 mg/l. A network-specific quality assurance programme was used to ensure uniformity of data.

The geometric mean MIC of penicillin was determined for each year, for serogroups B and C meningococci and for patients known to have survived or died. Tests of significance were performed by χ^2 analysis.

RESULTS

A total of 1434 isolates from cases of IMD was examined by the standard MIC method in the 6 years from 1994 to 1999. These included 874 serogroup B strains and 501 serogroup C isolates which together totalled about 96% of all meningococci examined. Serogroups W135 and Y comprised most of the remaining isolates. Specifically, there were no serogroup A strains. The numbers of meningococci tested in each year ranged from a low of 167 in 1994 to a high of 310 in 1997 (Table 1). A large number of serotypes and serosubtypes, especially those of serogroup B, was present in each year (data not shown).

The proportion of penicillin susceptible strains decreased from 45% in 1994 to 34% in 1995 and to 27% in 1996. Subsequently this percentage ranged between 19 and 26% ($P\leqslant0.001$). Strains less sensitive to penicillin comprised all of the remaining isolates except for single strains with an MIC of 1 mg/l in each of the years 1996 and 1999. The geometric mean MIC of penicillin increased from 0.045 to 0.065 mg/l from 1994 to 1999. No significant difference in geometric mean penicillin MICs was noted between serogroup B and serogroup C isolates.

Outcome data on 901 cases were available. Of these, 825 survived and 76 succumbed to IMD. From those who survived, 201 (24.3%) of the isolates were susceptible and 624 (75.7%) less susceptible to penicillin. From those with fatal outcomes, 32 (42%) of isolates were susceptible and 44 (58%) less susceptible to penicillins ($P<0.001$). The geometric mean penicillin MIC of isolates from those who survived was 0.06 mg/l and of those who died 0.048 mg/l.

All isolates were fully susceptible to ceftriaxone. Two isolates in each of the years 1996 to 1999 inclusive had MICs of 1 mg/l of rifampicin and a single isolate in 1999 had an MIC of >1 mg/l. All isolates were quinolone susceptible with the exception of a single isolate in 1998 with a ciprofloxacin MIC of 0.25 mg/l.

DISCUSSION

A significant shift in the susceptibility of invasive meningococci to the penicillins occurred in Australia between 1994 and 1996 and was maintained until 1999. In the period under review the proportion of strains fully penicillin-susceptible declined from 45 to 26% and the geometric mean penicillin MIC showed a corresponding rise from 0.045 to 0.065 mg/l. Only two resistant isolates, both with penicillin MICs of 1 mg/l, were found amongst the total of 1434 strains tested by standard methods.

Despite the trend towards decreasing penicillin susceptibility in Australia in recent years, penicillins remain a suitable therapy for the treatment of IMD. However, it is very difficult to establish a precise relationship between penicillin MICs and disease outcome in IMD. Clinical and bacteriological treatment failure has been recorded in an adult case of meningococcal meningitis due to an isolate with a benzylpenicillin MIC of 0.64 mg/l when the dose was 500 000 units 6 hourly.[19] In a small series, children with infections with less susceptible strains experienced a higher complication rate.[20] In contrast, a case–control study in children found no difference in outcome between groups infected with susceptible and less susceptible strains.[21]

In the sample reported here, penicillin susceptibility or resistance in meningococci isolated from cases of IMD could not be shown to be related to disease outcome. A similar finding has been previously reported from Spain.[22] Paradoxically, in those patients infected with strains less susceptible to penicillins, the proportion that survived was significantly higher than those who survived infection with fully susceptible strains. In addition, the geometric mean MIC for penicillin of isolates from the survivor group was also higher than that for meningococci from the fatal group. Further, both isolates with MICs of 1 mg/l were obtained from patients who survived IMD. This suggests that other and recognised factors which influence survival in IMD, such as host response and immunity, time to diagnosis and treatment, available supportive measures and organism virulence, are more relevant to survival than the decrease in penicillin susceptibility observed here. There were no data to measure the relative frequency of sequelae of IMD caused by susceptible and less-susceptible meningococci. Importantly, information was not generally available on the antibiotic treatment regimen used for IMD in individual cases. Experience would also suggest that therapy for IMD

is not restricted to the penicillins in Australia. These additional confounding factors make it difficult to assess the impact of antibiotic resistance on the outcome and sequelae of IMD under present circumstances. Additionally, most cases of IMD in Australia are sporadic in nature and are caused by diverse serogroup B and C phenotypes.[23]

There have been suggestions overseas that infections with serogroup C meningococci have a worse outcome compared with those with serogroup B organisms.[24] The data published by the NNN derived from its sample of invasive meningococci indicate that a similar situation exists in Australia.[23] However, when strains from the same sample were examined here, there was no difference in the penicillin susceptibility of serogroup B and C meningococci. This suggests that the differences in survival in IMD caused by the two serogroups would be due to factors other than penicillin susceptibility.

Any *in vitro* resistance to the other antibiotics commonly used for prophylaxis and treatment of IMD in Australia was infrequent and sporadic. All isolates were fully susceptible to ceftriaxone and by extension to other third-generation cephalosporins. A small number of isolates had altered susceptibility to rifampicin, but only a single isolate possessed high level resistance. A single strain exhibited low level quinolone resistance. Full particulars relating to this isolate have been previously published.[16]

The importance of continuing surveillance to establish not only the proportion of strains with altered susceptibility at any one time but also trends in antibiotic susceptibility was reinforced in this study. This is best achieved using continuing surveillance by reproducible and standard techniques. The MIC data reported here were derived from laboratories participating in a long-term monitoring programme using established methods, and comparability of data was further verified by a quality assurance programme. Numeric MIC values derived by different testing procedures are not usually comparable as use of different test methods may produce substantial variations in MIC values.[22,25]

While the above findings suggest that antibiotic resistance in *N. meningitidis* is not yet a major problem in Australia, it would seem prudent to continue to monitor the antibiotic susceptibility of meningococci. It is known that meningococci have the capacity to acquire resistance by mechanisms already elucidated in *Neisseria gonorrhoeae*, albeit much less rapidly. The lessons learned from the emergence and spread of gonococcal resistance can be applied, in part at least, to meningococci given the high degree of DNA homology between the two organisms. Such data are important not only for individual patient management with therapeutic antibiotics but also for public health aspects of IMD control by means of chemoprophylaxis.

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