

## Serogroups and antibiotic susceptibility patterns of *Neisseria meningitidis* isolated from army recruits in a training camp

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### Abstract

Invasive *Neisseria meningitidis* infection is rare but carries a high mortality rate. The carriage rate in the normal population is around 10% and can be higher in confined populations. A study on the prevalence of carriage of *N. meningitidis* was conducted among 3195 army recruits after 2 months of intensive training in an army camp. *N. meningitidis* was isolated from 37.0% of these recruits. Two hundred and ten of *N. meningitidis* isolates were subjected to serogrouping and 100 to antibiotic sensitivity testing by the disc diffusion method and E-test for penicillin. Ten (4.8%) of 210 *Neisseria meningitidis* serogrouped belonged to serogroup W135, 3.33% serogroup A and 81.4% belonged to either serogroup X, Y or Z. With the agar disc diffusion method, all the *N. meningitidis* showed susceptibility to chloramphenicol, rifampicin, cefotaxime and levofloxacin; 85% of the strains were resistant to cotrimoxazole and 12.5% resistant to penicillin. However, based on minimum inhibitory concentration, none of the *Neisseria meningitidis* tested was resistant to penicillin.

*Key words: Neisseria meningitides, serogroups, sensitivity pattern*

### INTRODUCTION

*Neisseria meningitidis* is one of the major causes of bacterial meningitis and community acquired septicemia throughout the world especially among children.<sup>1</sup> Meningococci have the capacity to spread rapidly from person to person, usually in relatively confined populations of young people such as college students or military recruits.<sup>2,3,4</sup> The only natural host of meningococcus is man. The meningococcus has its natural habitat in the mucous membranes of the oropharynx of normal human hosts. Those harboring the organism are usually asymptomatic and are presumably the major source of transmission of pathogenic strains. Carriage of low-virulence strains may be beneficial because it leads to the development of bactericidal antibodies, but at the same time, the transmission of pathogenic strains represents a risk for the general population. During periods of endemic disease asymptomatic carriage rates in a semi-secluded population, such as military recruits, may exceed 50%.<sup>3</sup>

We conducted a survey among army recruits undergoing intensive training in an army training camp to determine the prevalence of *N.*

*meningitidis* carriage, its serogroup distribution and antibiotic susceptibility pattern in a confined population of healthy young adults in Malaysia.

### MATERIALS AND METHODS

#### *Specimens*

Throat and anterior nasal swabs were collected from army recruits undergoing intensive training in an army camp from April to October 2005. Sampling was done for 3 weeks duration in June, during the third month of their residence in the camp. They were also requested to answer a set of questions related to their biodata and epidemiology as well as recent medical history. Samples were inoculated directly onto modified Thayer Martin (MTM) medium and transported to the Institute for Medical Research (IMR) in a container containing reduced oxygen content (resemble candle jar). All *Neisseria meningitidis* isolates were identified by standard bacteriological methods, including Gram stain, oxidase and aminopeptidase activity, and carbohydrate degradation tests.

### Antibiotic susceptibility testing

One hundred isolates were subjected to antibiotic susceptibility testing based on the almost 100% susceptibility of *N. meningitidis* to commonly used antibiotics where the minimum significant number for testing was 60-80 isolates. The antibiotic susceptibility patterns of 100 *Neisseria meningitidis* strains to cotrimoxazole, chloramphenicol, rifampin, levofloxacin, cefotaxime and penicillin were determined by a disc diffusion method using Mueller Hinton II agar with 5% sheep blood. The table used for interpretation of disc diffusion results was that recommended by the Clinical And Laboratory Standards Institute (CLSI) 2005<sup>5</sup> for *Neisseria gonorrhoeae*. Strains resistant to penicillin by disc diffusion were subjected to minimum inhibitory concentration (MIC) determination using E-Test strips obtained from AB Biodisk, Solna, Sweden, according to the manufacturer's instructions. The MIC interpretation was based on breakpoints for *N. meningitidis* in the same CLSI document. Isolates were classified as penicillin less susceptible or intermediate if the MIC exceeded 0.06µg/ml and penicillin resistant if the MIC was ≥0.5µg/ml. *Streptococcus pneumoniae* ATCC 49619 was used as the quality control organism and was included each time a set of isolates was tested.

### Serotyping

The serogroups of randomly selected 210(17.9%) of the 1181 *Neisseria meningitidis* isolates, were determined by slide agglutination with polyclonal sera obtained from DIFCO laboratories, Detroit Michigan, USA.

## RESULTS

A total of 3195 army recruits who registered for an intensive training course in an army training camp were screened. They were young adults between 17 to 24 years of age with a majority of 61.1% in the 18-20 year age group. 11.3% were female. Malays represented 85.5%, 10.0% were natives from East Malaysia and the rest were other races including Indian and Chinese.

A total of 6390 samples, both throat and nasal swabs from each recruit, were obtained and inoculated onto modified Thayer Martin (MTM) agar. *Neisseria meningitidis* was isolated from 100 (3.13%) of 3195 nasal swabs and 1099(34.40%) of 3195 throat swabs. Positive isolates were obtained from 1181 personnel thus giving a carriage rate of 37.0%. Of the

1181 recruits 6.0% were female recruits which giving a carriage rate among females of 19.7%. The carriage rate among male recruits was 39.15%.

All strains subjected to antibiotic sensitivity testing were susceptible to chloramphenicol, rifampin, levofloxacin, cefotaxime and penicillin. Ten *meningococcal* strains (12.5%) were less susceptible to penicillin by disc diffusion but all fell within the susceptible category based on the MIC of ≤ 0.064µg/ml measured by E-Test. The MICs ranged between 0.023µg/ml and 0.064µg/ml. The rate of resistance to cotrimoxazole was 84%. (Table 1.)

The majority (81.0%) of the 210 *Neisseria meningitidis* strains subjected to serogrouping, belonged to either X, Y or Z (171 strains). Ten (4.7%) strains were serogroup W135 and 3.3% (7 strains) serogroup A. Ten strains (4.7%) were untypable because of autoagglutination while 11 (5.2%) other strains were not reacting with the available antisera. Two strains (0.9%) cross-reacted with all the available antisera and were therefore also not typable (Table 2).

## DISCUSSION

About 37.0% of the recruits were colonized by *N. meningitidis* which is more than 3 times more than the normal population of Gloucestershire UK where the overall meningococcal carriage rate was 10.9%.<sup>4</sup> This carriage rate is slightly low compared to other studies among military recruits where the carriage rate was more than 50%.<sup>6</sup> This could be due to the exposure to antibiotics prior to sampling. From the questionnaire, the majority gave a history of taking antibiotics for fever and various types of minor respiratory illness such as coughs and colds during the 2 months prior to the sampling. However this is significantly higher than the estimated carriage among the normal population which is about 10% at any given time.<sup>3</sup>

Male carriers outnumbered female carriers of meningococci by 2:1, slightly more than the survey by Cartwright et.al<sup>4</sup> which observed a 3:2 ratio.

Various serogroups were detected including A (3.3%) and W135 (4.7%) and 171 (81%) were either serogroup X, Y or Z. About 11% of the isolates were non-serogroupable due to the limited number of antisera available. Our finding agrees with that of others that serogroups X, Y and Z are common serogroups carried by the normal population.<sup>1</sup> However we had more

**TABLE 1: Sensitivity patterns of *N. Meningitidis***

Antimicrobial	Results of agar disc diffusion			No. of isolates
	R	I	S	
Cotrimoxazole	84	-	15	99
Chloramphenicol	-	-	99	99
Rifampin	-	-	99	99
Levofloxacin	-	-	99	99
Cefotaxime	-	-	99	99
Penicillin*	-	-	90	99
<b>Penicillin MIC of 10 isolates showing reduced susceptibility in agar disc diffusion test:</b>				
		MIC	Number	
		0.023 µg/ml	1	
		0.032 µg/ml	1	
		0.047 µg/ml	4	
		0.064 µg/ml	4	

\* Susceptibility based on disc diffusion and MIC. S=Sensitive, I = Intermediate, R=Resistant.

serogroups A and W135. Serogroup A caused sporadic cases in Malaysia while serogroup W135 was introduced to the country by pilgrims returning from the Haj following the outbreak among the pilgrims in Mecca in 2000.<sup>7,8,9</sup> This is consistent with the increasing prevalence of W135 serogroup in many parts of the world.

Resistance to trimethoprim-sulphamethoxazole was high among these isolates and the rate was almost similar to other reports.<sup>10,11</sup> The widespread resistance to this antibiotic is possibly due to the early introduction of sulphonamides. All the isolates subjected to sensitivity testing were still very sensitive to other commonly used antibiotics. However the MIC of

penicillin in 4 of 10 isolates tested is at the higher end of the susceptibility range. The high rate of colonization by penicillin intermediate strains has been reported elsewhere.<sup>10</sup> High penicillin MIC levels among clinical isolates has previously been reported in Malaysia.<sup>12</sup> Continuous surveillance of the penicillin susceptibility of meningococci should be encouraged in order to detect the emergence of penicillin resistant strains which could complicate the treatment of meningococcal diseases. However, as in other studies<sup>10,11,13</sup> the alternative agents for treatment such as broad spectrum cephalosporines and quinolones demonstrated excellent *in-vitro* activity against meningococci in this study.

**TABLE 2: Serogroups of *N. Meningitidis***

Serogroups	Number (%)
W 135	10 (4.74)
X-Z	171 (81.04)
A	7 (3.32)
Other than W 135, A-D, X-Z	11 (5.21)
Auto agglutination	10 (4.74)
Untypable	2 (0.95)
<b>Total</b>	<b>211 (100)</b>

## Conclusion

Transmission of *Neisseria meningitidis* among army recruits is high leading to a high carriage rate. Colonisers belong to various serogroups including serogroups that are commonly associated with invasive infection. As new army recruits are subjected to both physical and mental stress during their training period they may become more susceptible to infection. For vaccine preventable diseases like meningococcal diseases, mass vaccination should be considered before the admission of these recruits to the camp. Although the prevalence of antibiotic resistance in meningococci is still low, antibiotic resistance surveillance should be done to monitor trends and to guide empirical treatment in the event of outbreaks. This is particularly important in Malaysia where the MIC levels for penicillin are now in the higher end of the susceptible range.

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