

NEISSERIA MENINGITIDIS STORAGE IDENTIFICATION AND MIC DETERMINATION

Pages with reference to book, From 177 To 179

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Abstract

The significance Minimum Inhibitory Concentration (MIC) of determination for Meningococci is described and according to the present study a shift in the sensitivity of Meningococcus is occurring, local isolates show higher Minimum Inhibitory Concentration for Chloramphenicol and Azactam. Strains of Meningococcus can successfully be stored in a domestic freezer (JPMA 39: 177, 1989).

INTRODUCTION

Neisseria meningitidis is an extremely intriguing organism. On one hand it inhabits the upper respiratory tract and other mucosal surfaces of humans, the only known reservoir, without causing any ill effects. On the other hand it causes invasive infection, killing the patient in few hours. Meningococcal disease tends to occur in clusters and usually in epidemic forms. Epidemics due to *N. meningococcus* group A tend to occur in cyclic waves approximately every decade and have been documented since the beginning of 20th century¹. In Karachi the first documented outbreak of *N. meningococcus* occurred during 1966-67. Unfortunately the group causing the outbreak is not known while the recently concluded epidemic was caused by sero group A and interestingly it followed identical steps in its spread. The isolates were sensitive to the routine drugs². *N. meningococcus* is closely related to *N. gonorrhoeae* and the difference is very subtle³. Both members of the family were highly sensitive to Penicillin but with passage of time one member *N. gonorrhoeae* become gradually resistant to Penicillin⁴ and according to one report, *N. gonorrhoeae* had the ability to inactivate Penicillin even in 1945⁵; the time Penicillin was commercially available yet *N. meningitidis* a sister member has failed to acquire this ability. It is universally accepted that *N. meningitidis* is sensitive to Penicillin and Chloramphenicol and as the isolates in the recently concluded epidemic were all found to be thus sensitive tested by disc sensitivity method, we decided to compare the MIC of local isolates because of considerable abuse of antibiotics compared to foreign isolate where use of antibiotic is restricted.

MATERIALS AND METHODS

Organisms:

A total of 22 clinical isolates of *N. meningitidis* were used for the determination of Minimum Inhibitory Concentration (MIC). They included 5 strains isolated in UK and provided by Dr Abbott, 9 strains were isolated by our laboratory and 8 strains were provided by "The Lab" and "Sind Lab", Karachi. All the local isolates belonged to Sero-group A. The UK strains were freeze dried. They were grown on chocolate agar identified and confirmed by gram's staining, oxidase and carbohydrate fermentation reaction. All local strains, as and when isolated, were confirmed, sensitivity test carried out by Kirby Bauer method and 10 colonies were emulsified in 1 ml of sterile tryptone soya broth (oxid) containing 10% glycerol, stored in the freezer compartment of refrigerator at -15 to -20°C. When needed, the tubes containing the organisms were thawed, subcultured onto chocolate agar, incubated at 36-37°C in a carbon dioxide incubator with enhanced humidity and 24 hour growth was used for

determination of MIC.

Determination of Minimum Inhibitory Concentrations:

Agar dilution methods were used for the determination of MIC. Media: The basal media used was Difco G.C. agar base with defined supplement⁶.

Carbohydrate Fermentation Reaction:

Carbohydrate fermentation reactions were determined by incorporating 1% carbohydrate in the medium and 1 ml of 0.02% Phenol red indicator sterilized by autoclaving at 10 lbs/sq inch (115°C) for 15 minutes and plates were poured.

Determination of Minimum Inhibitory

Concentrations:

Agar dilution method was used for the determination of MIC. A range of concentration of Penicillin, Chloramphenicol, Azactam and Amikacin were prepared according to the recommendations⁷ to give the required final concentration as given in the Table.

TABLE. M.I.C of local and international N. Meningitidis isolates in ug/ml

Origin	Total tested	Penicillin					Chloramphenicol				Azactam			Amikacin							
		0.02	0.05	0.1	0.2	0.4	0.5	1.0	2.0	3.0	4.0	0.5	1.0	2.0	3.0	4.0	1	2	4	6	8
Local	17	16	1	-	-	-	10	4	1	1	1	13	3	1		NIL	2	NIL	3	5	7
	% Sensitive	94.1	5.8				58.8	23.5	5.8	5.8	5.8	76.4	17.6	5.8		NIL	11.7	NIL	17.6	29.4	41.1
International	5	5	-	-	-	-	4	1	-	-	5	-	-	-	-	NIL	1		1	2	1
	% Sensitive	100					80	20			100					NIL	20	-	20	40	20

Plates were poured and used within a week of preparation.

Inoculation of Plates:

A multipoint inoculator (Denley Instruments Ltd, Billingham, Sussex, United Kingdom) was used to deliver 0.001 ml inocula (10⁶CFU) to the surface of antibiotic containing plates, carbohydrate containing plates and control plates without any antibiotics or carbohydrate were also inoculated as growth control. After inoculation, the carbohydrate plates were cut by using sterile scalpel blade in order to avoid smudging of fermentation results. All plates were incubated at 36°C in an atmosphere of 5% carbon dioxide with enhanced humidity. Results were recorded after 24 and 48 hours and the MIC's were taken as the lowest concentrations of antibiotic preventing the growth. Carbohydrate fermentation were recorded by observing for growth and change in colour from red to yellow.

RESULTS

Storage Results:

All the strains of N. meningitidis stored in freezer survived for over 6 months. This provides a simple, convenient and economic method for preserving cultures where sophisticated facilities are not available.

Carbohydrate Fermentation:

All the strains tested fermented Glucose and Maltose while Sucrose and Lactose were negative and the results fully matched with fermentation reactions compared with serum free sugars.

MIC Determination:

On disc sensitivity all strains were sensitive to Chloramphenicol, Penicillin and Azactam. The results of MIC are recorded in the Table and it is interesting to note that there is a difference between the United Kingdom isolates and local isolates as far as MIC are concerned. In all cases, local isolates show an increased resistance, with Penicillin it is only marginal 5.8% (1 strain) has MIC of 0.05ug/ml, with chloramphenicol 17% isolates shows MIC greater than 1 ug/ml, with Azactam it amounts to 23% and in the case of Amikacin more than 41% local isolate has MIC > 8 ug/ml as opposed to 20% of UK isolates.

DISCUSSION

In spite of the close relationship enjoyed by *M. meningitidis* with *N. gonorrhoeae*, *N. meningitidis* has failed to acquire the ability to produce Beta lactamase but if this ever happens it would be devastating. One way would be to carry out MIC on meningococcal isolates specially in the parts of the world where antibiotic abuse is a common practice and all laboratories in the developing world carry out antibiotic sensitivity tests by Disc diffusion method. The study involves a limited number of isolates but reveals that the sensitivity pattern of a Meningococcal isolates is exhibiting a gradual shift towards resistance to the commonly used antibiotics, Penicillin and Chloramphenicol. the increase in MIC for Chloramphenicol is higher as compared to Penicillin. Organisms can be stored conveniently in domestic refrigerators for appreciable length of time and carbohydrate fermentation reactions carried out using a multipoint inoculator. The abuse of antibiotics, specially underdosing, may have the clue for the gradual shift of MIC in Meningococci.

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