JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1980, p. 633-635
0095-1137/80/10-0633/03$02.00/0

Vol. 12, No. 4

Extracellular Neuraminidase Production by Clinical Isolates of Group B Streptococci from Infected Neonates

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A total of 73 clinical isolates of group B streptococci obtained from diseased infants in 23 states and Puerto Rico were examined for extracellular neuraminidase production. The association of elevated levels of neuraminidase with serotype III isolates was evident in a broad geographical distribution.

A previous study indicated that serotype III isolates of group B streptococci (GBS) from diseased infants in the Houston, Texas, area were more often producers of elevated levels of extracellular neuraminidase than type III isolates from asymptomatically colonized infants (2). Furthermore, only low levels of neuraminidase were associated with isolates of GBS from the other serotypes (Ia, Ib, Ic, and II). These results suggested that serotype III isolates were unique in their ability to produce high levels of the enzyme. The present study was undertaken to examine the association of neuraminidase levels with isolates of GBS from infected infants in a representative sampling of the United States.

Cultures from infected infants in 23 states and Puerto Rico were obtained through the courtesy of Richard Facklam, Center for Disease Control, Atlanta, Ga. The storage of bacterial strains, culture medium and growth conditions, preparation of culture filtrates, and enzyme assay were performed as previously described (2). A total of 73 strains representing all five serotypes were examined. Type III isolates from infected infants in 18 states (Table 1) could be divided into two distinct categories: high-neuraminidase-producing (> 140 nmol of sialic acid released per mg of cell dry weight) and non-neuraminidase-producing strains (≤ 10 nmol of sialic released per mg of cell dry weight). In this study 22 of 39 (56%) type III isolates were high producers of neuraminidase with no difference in production between isolates from blood or cerebrospinal fluid. In contrast, non-type III GBS isolates (Table 2) from infected infants in 18 states and Puerto Rico were, with few exceptions, low producers of extracellular neuraminidase (> 10 ≤ 140 nmol of sialic acid released). One type Ib isolate was classified as a nonproducer, and six of seven strains which share the type II and Ic antigenic determinants (5) were also non-neuraminidase-producing strains. These results confirmed previous observations and established a quantitative pattern for neuraminidase production by the various serotypes of GBS on a broad geographical basis.

Combined data from the previous study (2) and the present report are presented in Table 3. Although the majority of type III isolates from infected infants (42/65) produced elevated levels of neuraminidase, there was not an absolute requirement for enzyme production to result in disease in the neonate. Other factors, in addition to neuraminidase, such as the antiphagocytic type III polysaccharide capsule (1), the size of the infecting dose, the immunological status of the neonate, and other possible virulence factors, probably contribute in combination to establish infection in the newborn. However, the association of elevated levels of neuraminidase with serotype III strains compared to non-type III strains was significant at $P \ll 0.005$ (Table 3). These results indicate that GBS strains possessing the type III-specific capsular polysaccharide can be subdivided into two major physiological categories based on neuraminidase production.

It is well documented that serotype III strains cause the great majority of late onset disease in the neonate (4); however, it is not known when and from what source the infant is colonized. Since all type III strains appear to be either high

TABLE 1. *Neuraminidase activity in 39 isolates of type III GBS from infected infants in 18 states*

Strain no.	Total neuraminidase activity[a]	Source of isolate	Geographical location
232	<10	CSF[b]	Alaska
225	<10	CSF	California
251	<10	Blood	California
233	183.8	Blood	Colorado
240	182.0	Blood	Delaware
245[c]	272.3	Blood	Florida
230	217.3	Blood	Illinois
246	198.6	Blood	Illinois
263	225.6	CSF	Illinois
211	<10	Blood	Illinois
282	249.9	CSF	Louisiana
256	<10	Blood	Louisiana
261	<10	CSF	Louisiana
222	221.5	CSF	Maryland
297	243.6	Blood	Maryland
210	280.3	Blood	Massachusetts
301	187.0	CSF	Massachusetts
260	<10	Blood	Massachusetts
268	<10	CSF	Massachusetts
219	218.7	Blood	Michigan
269	239.3	CSF	Michigan
298	212.1	Blood	Michigan
293	164.4	CSF	Michigan
215	<10	Blood	Michigan
280	<10	CSF	Michigan
289	<10	Blood	Michigan
300	<10	Blood	Michigan
247	250.4	CSF	Minnesota
236	<10	Blood	Minnesota
290	<10	Blood	Mississippi
276	217.3	CSF	North Carolina
283	223.8	CSF	North Carolina
218	219.1	CSF	Oklahoma
275	177.5	Blood	Oklahoma
292	<10	CSF	Oklahoma
254	227.1	CSF	Pennsylvania
295	212.5	CSF	Rhode Island
221	<10	Blood	Tennessee
279	<10	CSF	Texas

[a] Cells were grown to late exponential phase in chemically defined medium (2) supplemented with 230 μg of human serum albumin per ml and 2% (vol/vol) Todd-Hewitt broth. Total activity is expressed as nanomoles of sialic acid released per minute per milligram of cell dry weight; average of duplicate determinations.

[b] CSF, Cerebrospinal fluid.

[c] Strain 245, classified as III/Ic.

TABLE 2. *Neuraminidase activity in 34 isolates of GBS other than type III from infected infants in 18 states and Puerto Rico*

Strain no.	Serotype	Total neuraminidase activity[a]	Source of isolate	Geographical location
277	Ia	66.9	Blood	Tennessee
284	Ia	42.7	Blood	Massachusetts
291	Ia	73.8	Blood	Massachusetts
299	Ia	53.1	CSF	Oklahoma
213	Ib	41.8	Blood	Delaware
216	Ib	<10	Blood	Hawaii
229	Ib	27.5	CSF	Illinois
286	Ib	44.1	CSF	Maryland
287	Ib	65.1	Blood	Michigan
294	Ib	51.6	Blood	Michigan
267	Ib	36.2	Blood	Oklahoma
272	Ib	29.6	Blood	Oklahoma
209	Ib	47.9	Blood	Virginia
270	Ib	63.1	Blood	Virginia
265	Ib	55.7	Blood	Texas
223	Ic	28.1	Blood	Massachusetts
238	Ic	63.1	Blood	Massachusetts
234	Ic	38.0	Blood	Maryland
244	Ic	57.8	CSF	Maryland
273	Ic	43.2	CSF	New York
235	Ic	31.3	Blood	Oklahoma
262	Ic	30.5	Lung biopsy	Texas
250	II	39.2	CSF	Connecticut
258	II	68.0	Lung biopsy	Minnesota
264	II	108.4	Blood	Louisiana
266	II	105.2	Blood	Puerto Rico
214	II/Ic	<10	Blood	Oklahoma
228	II/Ic	<10	Blood	Mississippi
231	II/Ic	55.2	Blood	Pennsylvania
257	II/Ic	<10	Blood	Louisiana
285	II/Ic	<10	Blood	Louisiana
288	II/Ic	<10	Blood	Michigan
296	II/Ic	<10	Blood	Vermont
237	Nontypable	34.9	Blood	Oklahoma

[a] Cells were grown and neuraminidase was assayed as described in Table 1, footnote *a*.

TABLE 3. *Clinical isolates of GBS from invasive neonatal disease*[a]

Serotype	No. of isolates with total neuraminidase activity[b] of:			
	>140	≤140–>10	<10	Proportion at ≥140[c]
III	42	0	23	42/65
Ia, Ib, Ic, II, II/Ic, or NT	3	47	9	3/59

[a] Classified by serotype and ability to produce extracellular neuraminidase. Isolates in the present study were combined with GBS from a previous report (2).

[b] Expressed as nanomoles of sialic acid released per milligram of cell dry weight.

[c] χ^2 Yates corrected = 44.8 ($P \ll 0.005$).

producers of neuraminidase or non-producers, the ability to produce this enzyme might serve as a useful tool in epidemiological studies, particularly when used together with a phage-typing system for GBS (3).

ACKNOWLEDGMENT

This work was supported by Public Health Service research grant AI 13836 from the National Institute of Allergy and Infectious Diseases.

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