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A Bacteriological Study of Meningitis in Pediatric Patients in Ahwaz,lran

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Summary

Of 164 cerebral spinal fluid samples received, 100 or 60.9% were from patients with suspected bacterial meningitis.

The positive culture rate for al 1 the samples was 24% or 40% if only the CSF samples from menigitis patients are included.

The most common cause of menigitis in the newborn was the gram negative bacilli.Etiological agents in infants from two months to three years included Hemophilus

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influenzae, Streptococcus pneumoniae, Neisseria meningitidis, other pyogenic cocci, and gram negative bacilli. Antibiotic studies showed that recommended combined drug therapy could probably be used in this area, until positive identification of the bacteria and results of the antibiotic sensitivity test are made available.

Introduction

Examination of the cerebral spinal fluid (CSF) is an invaluable aid in the evaluation of the patient with bacterial meningltis. Acute bacterial menigitis can generally be distinguished from other types of meningitis, such as viral, fungal and tubercular [1] ln most cases of acute bacterial meningitis, the gram stained sediment of the CSF reveals the etiological agent[1.,3.]. An exception to this is bacterial meningitis caused by Neisseria meningitidis, in which the smears and cultures may be negative[1]. When good laboratory services are available, chemical and cytological lab data should correlate with the findings from the bacteriological examination of the CSF.

This study reports the results of 164 CSF samples from pediatric patients. These samples were submitted to the Bacteriology laboratory for routine examination. One hundred of these samples were from patients with a diagnosis on admission to the hospital of meningitis.No other information about the illness was given at the time the sample was sent. it was not noted whether septic or aseptic meningitis was suspected. All of the samples were received from a pediatric ward in one of the Univer-

sity Hospitals in Ahwaz, Iran.

The main objective of this study was to determine the incidence of positive cultures from the CSF samples of patients with suspected acute bacterial meningitis. In addition to this, the prevalent causes of acute bacterial menigitis in pediatric patients were to be determined. it was also of interest to evaluate the antibiotic sensitivity of the bacteria from the CSF samples and to possibly make recommendations for the best approach to recovery of the organisms and for treatment of these cases on the basis of the agent and its sensitivity to antibiotics in vitro.

Materials and Methods

The CSF samples were collected by the staff of the pediatric Department. Approximately one ml of the CSF was aseptically placed into 10 ml sodium thioglycollate broth (Difco Co.)that contained 1% dextrose. It was requested that 0.5 ml of the CSF should be placed in a sterile tube for staining. However, in most cases this was not done, and only the CSF sample already inoculated to thioglycollate was received by the Bacteriology lab. This eliminated the possibility of doing a direct stain on the CSF sample. The samples were taken from the ward to the lab as soon as possible. If there was to be any delay in transport, the inoculated samples were stored at room temperature and the CSF samples were stored at 4 C.

Laboratory procedures: the CSF samples were centrifuged(2,500 rpm for 15 minutes) and the slides from the

sediment were stained by Grams, Zeil-Neilson, and India Ink mehtods. Inoculated cultures were also stained by these methods as soon as they arrived at the laboratory, Subcultures were made to two sets of enriched media (Chocolate agar with Isovitalex Enrichment, BBL Co.,and 5% sheep or human blood agar), Incubation was at 35-36 C with one set of the plates in 5-10% CO_2 and the other set in a BBL-Gas Pak Anaerobic system. Cultures were subcultured in the same manner every 48 hours and gram staining of the thioglycollate broths was repeated. If no growth occurred, cultures were kept for seven days before being discarded.

Bacteria were identified by routine laboratory methods[3.,4.]. Sensitivity tests were done using a standardized disc method[5]. Positive and negative controls were used for all media, stains and reagents.

Results

A total of 164 CSF samples were received for bacteriolaogical study over a period of nine months (January-August, 1977). The admission diagnoses of the pediatric patients as given on the report form that accompanied each sample, are listed in Table 1.. Only 100 (60.9%) of the patients were suspected to have meningitis. Of these 100 patients, it is not known what per centage of the CSF samples resulted in negative cultures due to the presence of aseptic forms of meningitis. Usually the culture is positive in cases of acute bacterial meningitis[1.]. However, many labs fail to recover a high per centage of the organisms due to poor transport

methods, delays in culture, as well as other reasons. Fourty (40%) of the CSF samples were positive for the presence of bacteria but only 39 were positive for bacterial growth after subculture. These samples were from the patients with an admission diagnosis of meningitis. lf all of the samples that were received are considered

Table 1.

Admission Diagnoses of 164 Pediatric Patients

Diagnosis	Number	Per Cent
Meningitis	100	60.9
Sepsis	22	13.4
Pneumonia,Respiratory lllness	17	10.4
Other (Gastroenteritis, jaundice, Fever, etc.)	25	15.3

(164), then the positive culture rate was 24%.

Although sterile tubes and instructions were provied for the collection of the CSF, most of the CSF samples were sent to the laboratory in the thioglycollate medium alone. ln only ten of the culture positive cases, the CSF was sent so that stains could be done. All ten slides were positive after gram staining. ln one case the slides was positive but the culture failed to grow.

The organisms that were isolated are shown in the order of prevalence in Table 2.. The most common isolates were Streptococcus pneumoniae, Staphy lococcus aureus, Escherichia coli, Staphylococcus epidermidis, Hemophilus influenzae, and Neisseria meningitidis. it is not known if the isolates of Staphylococcus epidermidis represented actual cases of meningitis. These patients also had positive blood cultures with Staphylococcus epidermidis but other clinical data was unavailable. The isolates of Staphylococcus epidermidis from CSF were more resistant to antibiotics and differed biochemically from Staphylococcus epidermidis strains isolated from the skin of patients or from fomites in the ward (uppublished data) Concerning the overall recovery rate of onganisms from the CSF samples, if these cases are omitted as possible contamination, the positive culture rate from all CSF samples received would be 21% or 35% if only CSF samples from suspected cases of meningitis are considered.

When the data was analysed according to the age of the patient, a change in the distribution of the bacterial isolates was seen, as shown in Table 3..This distribution is similar to other reports of variation in etiological agents of bacterial meningitis in relation to the age of the patient[1.,2.,6.]. Blood cultures from the newborn to one month old patient group were positive in 64% (7) of the cases wich were caused by Escherichia coli, Staphylococcus epidermidis, and Klebsiella pnuemoniae (unpublished data).

The sensitivity of the isolates from the CSF samples to Ampicillin, Penicillin, Methicillin, Chloramphenicol and Gentamicin in vitro, is shown in Table 4.. The only

antibiotic to which all the isolates were sensitive was Gentamicin.

Clinical data was retrospectively obtainable on only 13 of the patients that had a positive CSF culture.This data is shown in Table 5.

Table 2.

Bacteria lsolated From Pediatric Meningitis Cases

Oragism	Number	Per Cent
Streptococcus pneumoniae	8	20
Staphylococcus aureus	8	20
Escherichia coli	7	18
Hemophilus influenzae	5	13
Staphylococcus epidermidis	5	13
Neisseria meningitidis	3	8
Streptococcus Group B	1	2
Streptococcus Group D	1	2
Flavobacterium meningiosepticum	1	2
Klebsiella pneumoniae	1	2
Total	40	

Table 3.

Etiological Agents of Bacterial Meningitis in Relation to the Age of the Patients

Age	Organism	Number	Per Cent
Newborn to One Month	Escherichia coli	4	37
	Staphylococcus aureus	2	18
	Staphylococcus epidermidis	2	18
	Streptococcus Group B	1	9
	Streptococcus pneumoniae	1	9
	Klebsiella pneumoniae	1	9
	Total	11	
Over One Month to One Year	Hemophilus influenzae	3	27
	Escherichia coli	3	27
	Streptococcus pneumoniae	2	19
	Neisseria meningitidis	1	9
	Staphylococcus aureus	1	9
	Staphylococcus epidermidis	1	9
	Total	11	
One To Three Years	Streptococcus pneumoniae	4	33
	Streptococcus aureus	3	25
	Staphylococcus epidermidis	2	18
	Hemophilus influenzae	1	8
	Neisseria meningitidis	1	8
	Flavobacterium	1	8
	Total	12	
Over Three Years	Staphylococcus aureus	2	33
	Streptococcus pneumoniae	1	17
	Streptococcus Group D	1	17
	Hemophilus influenzae	1	17
	Neisseria meningitidis	1	16
	Total	6	

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Table 4.

Per Cent Sensitivity of Bacteria From Thirty-nine Pediatric Meningitis Cases to Some Antibiotics

Organism	Number Isolated	Per Cent Sensitive To Antibiotics				
		AM*	P	MC	C	GM
Staphylococcus aureus	8	25	13	38	75	100
Strept.pneumoniae	8	100	100	100	100	100
Escherichia coli	7	43	0	0	0	100
Hemophilus influenzae	5	100	60	100	100	100
Staph.epidermidis	5	20	40	40	80	100
Neisseria meningitidis	2	100	100	100	100	100
Klebsiella pneumoniae	1	0	0	0	0	100
Flavobacterium	1	100	100	100	100	100
Streptococcus Group B	1	100	100	100	100	100
Streptococcus Group D	1	0	0	0	0	100

*AM=Ampicillin, P=Penicillin, C=Chloramphenicol, MC=Methicillin, and GM=Gentamicin

Table 5.

Some Clinical Data On Thirteen Pediatric Patients With Acute Bacterial Meningitis

	Age	Organism Isolated	Clinical Signs	Previous Therapy	Hospital Therapy/Days	Death
35	de	N.meningitidis	Projected Fontanelle	none	Ampicillin/7da	----
44	de	Escherichia coli	Proiected Fontanelle Weak, Fever,Stiff Neck	yes	Ampicillin Gentamicin/10da	----
4	mo	Escherichia coli	Open Superior Fontancelle Chills, Fever, Weak	none	Ampicillin Gentamicin/8da	----
5	mo	Staph. aureus	Fever, Chills,Stiff Neck	"	Ampicillin Gentamicin/14da	-----
5	mo	H. influenzae	Projected Fontanelle Fever, Chills, Weak	yes	Ampicillin Penicillin /21da	-----
6	mo	Escherichia coli	"	none	Ampicillin/1 de	on 1st day
10	mo	Pneumococcus	"	"	"	" " "
(2 patients)			"	"	" /2da	on 2nd day
1	yr.	H.influenzae	Fever, Chills,Stiff Neck	"	Ampicillin Gentamicin/1 da	on 1st day
1	yr.	Pneumococcus	"	"	Penicillin/28 da Chloramphenicol/3 da	------
2	yr	Staph.aureus	"	"	Ampicillin Gentamicin/14 da	------
10	yr	Staph. aureus	"	"	Ampicillin/6 da	on 6th day

Discussion

Of the 164 CSF samples sent from a pediatric ward to the Bacteriology laboratory, only 100 were from patients described as suspected meningitis cases. No other information was consistently provided. This data may reflect the fact that this study involved a University teaching hospital and that samples were collected as precautionary measures. Fourty of the samples were found to contain bacteria although one failed to grow in the laboratory. This sample that was positive for the presence of bacteria but did not grow after subculture, contained gram negative diplococci resembling Neisseria. The reasons for the negative culture could have been cell death during transport or that the media failed to support the growth of this strain even though quality control strains were able to grow.

This study shows that if cases of suspected acute bacterial meningitis are cultured, the causative agent and its antibiotic sensitivity pattern could be made availabe to the physician in 39% of the cases. Negative cultures (71%) may have resulted from some errors in methods of transport or lab procedure. It was not known in many cases if the patients had received antibiotic therapy before admission to the hospital, which could also have been a factor in the negative cultures from some of the samples (7,).

The most common cause of meningitis varied with the age of the patients. The variation is not different in the Ahwaz area from that reported from other areas (1.,

2.,6.). The cases of Escherichia coli meningitis occurred in a cluster at the same time and may have represented an outbreak on the ward.
No other time clustering was observed for any of the other isolates.

Gentamicin was the only antibiotic to which all the bacteria isolated from these CSF samples were sensitive in vitro. Ampicillin was effective in vitro against Streptococci, Hemophilus, and Flavobacterium but not against all of the Staphylococci, Klebsiella and Escherichia coli.
The choice of antibiotic therapy in bacterial meningitis depends on 1.) the pharmacological properties of the drug, 2.) the type of organism and its resistance, and 3.) the underlying conditions of the patient (2.).Gentamycin is an aminoglycoside which is usually a poor choice when given alone as the therapy for meningitis (1.,2.). Therefore, it has been recommended that in undiagnosed bacterial meningitis a combination of Gentamicin and Ampicillin be given to neonates and Ampicillin and Chloramphenicol be given to older ingants and children1.,2.,8.).

lt has also been suggested that when Ampicillin and Chloramphenicol therapy has been initiated, one drug should be discontinued when the antibiotic sensitivity of the causative agent is known(6.,8.). This action may reduce the unecessary side effects that may result from Chloramphenicol.
Due to the emergence of resistant strains of Hemophilus influenzae, Ampicillin is not recommended as single therapy unless the antibiotic sensitivity test results

are Known [9.,10.]. Although no resistant strains were isolated in this study,we cannot exclude their existance and should be on the alert for them in the future.

The results of this study have led to the following conclusions:1.) the incidence of positive bacterial cultures in suspected bacterial meningitis from pediatric patients was 39%; 2.) the prevalent causes of these cases of bacterial meningitis varied according age group and did not vary greatly from patterns seen elsewhere; 3.) according to even the small amount of clinical data that was made available, the use of the suggested combined drugs would be a satisfactory therapy until results of the actual sensitivity pattern of the agent is reported; and 4.) every effort should be made to improve the laboratory examination of CSF samples to provide accurate and prompt results. Direct inoculation on the ward of the CSF to thioglycollate medium and speedy transport to the lab are probably responsible for a high recovery rate in this study. However, providing a medium transport method on the ward led to failure to send even minute samples of CSF in sterile tubes which eliminated in mamy cases the important aspect of the direct smear. Greater cooperation on the part of the lab and ward could perhaps eliminate this problem.

Once at the laboratory, the CSF should be examined using standardized tests and media that are quality controlled to assure the accuracy of the report.

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