Diagnosis of Culture Negative Acute Bacterial Meningitis by Using A 16S rDNA - Based Gene Sequencing Directly from CSF Samples

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Abstract: Background: Acute bacterial meningitis is an important medical emergency with high morbidity and mortality rate all over the world. The uses of 16S rDNA amplification and sequencing have been proposed as a strategy for diagnosis especially in culture negative cases. **The aim of the study:** Was to evaluate the diagnosis of culture negative acute bacterial meningitis by using 16S rDNA-based gene sequencing applied directly on CSF samples, in order to identify the causative bacterial species for the proper choice of subsequent antimicrobial therapy. **Methodology**: CSF samples were collected from 30 patients expected to have acute bacterial meningitis (15 with expected community acquired meningitis & 15 with expected VP shunt related meningitis) and from 7 patients expected to have viral meningitis. Blood samples were also collected from patients with expected community acquired meningitis for blood cultures. CSF samples were subjected to macroscopic, chemical, cytological and bacteriological examination. DNA extractions from CSF samples were done using the QIAamp mini kit. The extracted DNA was amplified and sequenced by using MicroSeq500 16S rDNA Bacterial Identification kit and cycle sequencing. The sequences were compared with those available in National Centre for Biotechnology Information (NCBI) by using Basic Local Alignment Search Tool (BLAST). **Results**: CSF culture was positive only in 16.7% while 16S rDNA was positive in 66.7% of cases. In culture negative cases, 16S rDNA was positive in 60% of cases. The detected bacterial species in expected VP related meningitis were *E. coli, K. pneumoniae, S. epidermidis* and *P. aeruginosa* and in expected community acquired meningitis were E. coli, K. pneumoniae, S. pneumoniae and S. aureus. The detection rate of 16S rDNA varies according to the combination of different diagnostic methods used for selection of patients, including clinical manifestations, CSF parameters and microbiological findings. In case diagnosed by gold standard methods, the sensitivity of 16S rDNA was 87.5% and the specificity was 100%. **Conclusion** Direct detection and sequencing of the 1st 500 bp of 16S rDNA by using cycle sequencing can provide a specific, sensitive, early, rapid and non expensive method for improving the microbiological diagnosis of acute bacterial meningitis, especially after initiation of antimicrobial therapy and in culture negative cases. In spite of lacking bacterial isolation and sensitivity test, it could re-optimize the empirical use of antibiotics based on the identified bacterial species.

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1. Introduction

Bacterial meningitis is considered as an infection that present in the subarachnoid space. It is a potentially life threatening emergency with high mortality rate up to 30% **(1).**

The onset of acute bacterial meningitis is sudden with rapid progression, which usually occurs within hours to few days, however subacute meningitis caused by TB or fungal infection, has gradual onset and progression within days to weeks **(2).**

S. pneumoniae, N. meningitidis and *H. influenzae* are considered as the most common causative pathogens in acute bacterial meningitis. However, the cause varies by age and different underlying condition of the patients **(1).**

To reduce death and permanent neurological damage, rapid accurate diagnosis and treatment as much as possible are needed **(3).** The key of diagnosis is obtaining turbid CSF by lumbar puncture. Increasing pressure, polymorphs count, protein level and decreased CSF-blood glucose ratio are very important signs of acute bacterial meningitis **(4).**

Microscopic examination and subsequent culture of CSF are the gold standard methods for diagnosis of bacterial meningitis and subsequent choice of proper antibiotics therapy **(2)** However, the culture may be negative if bacterial concentration in the CSF is low or the number of viable organisms in the CSF is low and if the patients stated antibiotic therapy before lumbar puncture As well as, the results of culture are only available after 24 to 48 h and it may even take longer

time **(5,6)**. Furthermore, the culture might miss the diagnosis of bacterial meningitis in at least 13% of cases because of fastidious, slow-growing and unusual or rare organisms (**7**).

Latex agglutination test, direct immune fluorescence and ELISA for the detection of bacterial antigens in CSF are not recommended in the microbiological routine diagnosis because of their low sensitivity and specificity **(8).** Although, Latex agglutination testis rapid and providing results in less than 15 minutes, there is a strong decline in the sensitivity was noticed in patient with antibiotic pretreatment prior to lumbar puncture **(1).**

Several molecular tests are available independent of bacterial growth for rapid and accurate diagnosis, especially in emergency condition. These methods are promising for the examination of CSF samples obtained after initiation of antibiotics therapy. As the polymerase chain reaction (PCR) is available for diagnosis, it can detect only single specific organism, or by multiplex PCR which can detect few number of organisms per assay **(9).**

So, the broad-range bacterial PCR assays can detect large number of bacterial species in a single assay, especially those which are rare or unknown causative agents of bacterial meningitis **(10).**

These assays are depending on recognition of conserved regions of the gene coding for 16S rDNA which is present in all bacteria and contains species-specific variable regions that provide a reliable basis for identifying different bacterial species by using specific primers. Recently, they have been used as tools for diagnosis especially in culture-negative bacterial infections **(11).**

The MicroSeq 500 16S ribosomal DNA-based bacterial identification system (Applied Biosystems Division) is one of the commercial tests that allow rapid and accurate identification of bacterial pathogens based on the nucleic acid sequences of 16S rDNA. It could be applied either directly on CSF or on pure cultures. The first 500-bp of the 16S rDNA gene of the bacterial strain are amplified by PCR then sequenced and analysed by using public databases such as GenBank or National Center for Biologic Information (NCBI) **(12,13).**

Aim of the Work:

The aim of this study is to evaluate the diagnosis of culture negative acute bacterial meningitis by using 16S rDNA-based gene sequencing applied directly on CSF samples, in order to identify the causative bacterial species for the proper choice of subsequent antimicrobial therapy.

2. Material and Methods

This study was performed during the period from September 2016 to February 2018and was approved by the Ethics Committee of Faculty of Medicine, Tanta University, Egypt.

The study was carried out on two groups: group I (patients group) which included 30 patients expected to have acute bacterial meningitis and group II (control group) which included 7 patients who were expected to have viral meningitis.

Group I was further subdivided into 15 patients with expected community acquired infection isolated in Tanta Hospital of Infectious Diseases and 15 infants with hydrocephalus who were admitted to Neurosurgery Department of Tanta University Hospital with application of ventriculoperitoneal shunt (VP shunt).

Patients with VP shunt were under prophylactic antibiotic therapy for two weeks after shunt insertion and sampling was performed before antibiotic change and after removal of shunt following clinical expectation of meningitis. On other hand, patients with community acquired meningitis were subjected for sampling before starting antibiotic therapy. In cases where sampling was postponed until CT was performed, sampling was done after initiating antibiotics.

CSF samples were obtained by lumber puncture in cases with community acquired meningitis or by ventricular tapping in shunt related meningitis. The samples were transported to the Microbiology and Immunology department under complete aseptic technique and divided into 3 sterile tubes two of them were used for the traditional methods of CSF examination and the third one was stored at -80 for molecular examination.

Blood samples for blood culture were obtained by venipuncture under complete aseptic technique from only patients with community acquired meningitis, where blood culture is not included in diagnosis of shunt related meningitis.

All patients are subjected to detailed history taking, clinical assessment for signs and symptoms of meningeal irritation in both expected cases of community acquired and shunt related meningitis.

The collected CSF samples were subjected to macroscopic examination and total WBCs count then centrifuged at approximately 1000 g for 5–10 minutes. The supernatant used for glucose and protein measurement while the sediments were cultivated on sheep blood agar, chocolate agar plates in the presence of 5% CO2 (in candle jar) and MacConkey's agar for 24 -48 hrsfor bacteriological examinationas well as direct microscopic examination by Gram's stain.

DNA extraction

The stored tubes were subjected to DNA extraction by using QIAamp mini kit (QIAGENInc., California) according to the manufacturer’s protocol

for DNA purification. The eluted DNA was stored until used in PCR.

PCR amplification of 16S rDNA:

15 µl of the extracted DNA were examined by amplification of 500 bp to the 5' end of 16S rRNA gene. It was done using MicroSeq500 16S rDNA Bacterial Identification PCR kit (Applied Biosystems, Thermo Fisher scientific). The bacterial 16S r DN Aamplification was performed in a the rmocycler, Gene Amp® PCR System 9700 - Applied Biosystemsin 9600 emulation mode. The PCR products were examined by using 2% agarose gel electrophoresis.

Sequencing of 16S rDNA:

It was done using MicroSeq500 16S rDNA Bacterial Identification sequencing kit (Applied Biosystems, Thermo Fisher scientific) and the cycle sequencing was done by using a thermo cycler, Gene Amp® PCR System 9700 - Applied Biosystems. The product was purified by using Big Dye X Terminator Purification Kit (Applied Biosystems, Thermo Fisher scientific) and were analysed by Capillary electrophoresis automated instrument 3500 Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions.

Data analysis:

Sequences obtained were analysed with the GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) 16S Ribosomal DNA sequences for species or genus assignment. The highest identity was selected as the identified species or genus. The range of accepted identity result was 97%-99% **(14).**

Statistical analysis:

Qualitative variables were summarized with absolute numbers and percentage and quantitative variables with means and standard deviation (SD) or medians and interquartile range (IQR) (depending on their homogeneity). Continuous variables were compared using t Student or the Mann–Whitney U test when appropriate. We analyzed categorical data using the $\chi 2$ test or Fisher's exact test when indicated.

3. Results

In the present study, the age of patients group was ranged from 1 month - 67 years versus 6 months - 60 years in control group. As regarding sex, the meningitis was found to be more common in male than female in both patients and control group (18/12 and 5/2 respectively).

Regarding CSF examination, there was significant increase in turbidity, total WBCs count and protein level with significant decrease in glucose level in patients than control group as shown in table **(1)**.

In direct microscopic examination, 26.7% were positive in patients group versus 100% negative in control group and CSF culture was negative in 83.3% of patients group and positive in 16.7% versus 100% negative in control group.

As regarding 16S rDNA, it was positive in 66.7% of patients group out of them there were 15 patients were culture negative while 100% were negative in control group as shown in tables **(2, 3)**.

Comparison between positive and negative cases for 16s rDNA among patients with expected bacterial meningitis was shown in table **(4)**.

The comparison between 16S rDNA detection and CSF Culture results in patients with expected VP shunt related meningitis was shown in table **(5)**.

The bacterial species detected by 16S rDNA sequencing in patients with expected VP shunt related meningitis included *Escherichia coli*, *Klebsiellapneumoniae sub. Pneumoniae, Staphylococcus epidermidis* and *Pseudomonas aeruginosa* which represented 27.3%, 27.3%, 27.3% and 18.1% of the detected bacterial species respectively. The BLAST identity was 99%, 99%, 98% and 99% respectively.

CSF and blood culture were positive in only 13.3% of cases with expected community acquired meningitis. *S. aureus* was isolated from 1 cases and E coli was isolated from the other case.

The comparison between 16S rDNA detection and CSF Culture results in patients with expected community acquired meningitis was shown in table **6**.

The bacterial species detected by 16S rDNA sequencing in patients with expected community acquired meningitis included *Escherichia coli, Streptococcus pneumoniae, Staphylococcus aureus and Klebsiellapneumoniae sub. Pneumoniae* which represented 33.3%, 33.3%, 22.2% and 11.1% of the detected bacterial species respectively. The BLAST identity was 99%, 98%, 99% and 97% respectively.

In expected cases with community acquired meningitis, E. coli was detected in neonate and adults, *S. pneumoniae* was detected in infants, children, adolescents and young adults, whereas *Klebsiellapneumoniae sub. Pneumoniae* and *Staphylococcus aureus* were detected in adults only.

In expected cases with community acquired meningitis, no risk factors were detected in patients with S. *pneumoniae* infection while E. coli was detected in neonate, in patient with UTI and in cancer patient. *K. pneumoniae* was detected in diabetic patient, whereas *S. aureus* was detected in two patients, one had D.M & pneumonia and the other had D.M & liver cirrhosis.

As regarding the detection rate of 16S rDNA, it was different with different method used for diagnosis of bacterial meningitis such as clinical, CSF and microbiological diagnostic methods. The detection

rate was 66.7%, 76.9%, 71.4% and 87.5% in category A, B, C and D respectively as shown in table **7.**

The sensitivity and specificity of 16S rDNA were 87.5% and 100% respectively in patients confirmed to have bacterial meningitis by gold standard method as shown in table **8**.

Table (1): CSF profile in group I (patients group) and group II (control group):

CSF Examination			Patients N= 30	Control N=7	P-value
Aspect	Clear	N	0	7	
		%	0%	100%	
	Hazy	N	9	0	P1 <0.001***
		%	30%	0%	
	Turbid	N	21	0	
		%	70%	0%	
Total WBCs count/mm^3		Median (Range)	3000 (200–38400)	160 (100–200)	P2 <0.001***
		Mean ± SD	5329.83 ± 7628.75	155.71 ± 42.37	
Glucose (mg/dL)		Median (Range)	20 (3–50)	50 (45–75)	P3 <0.001***
		Mean ± SD	22.27 ± 12.13	56.43 ± 12.15	
protein (mg/dL)		Median (Range)	245 (65–480)	80 (65–100)	P4 0.003**
		Mean ± SD	237.73 ± 127.08	82.14 ± 14.68	
PMNLs%		Median (Range)	89.5 (50–95)	–	
		Mean ± SD	85.33 ± 11.48	–	
Lymphocytes		Median (Range)	–	90 (85–95)	
		Mean ± SD	–	90.29 ± 4.15	

Table (2):16S rDNA detection in group I (patients group) and group II (control group):

16S rDNA detection result		N	%
Patients (N=30)	negative	10	33.3
	positive	20	66.7
Control (N=7)	Negative	7	100%
	Positive	0	0%
P- value		0.002**	

Table (3): Comparison between 16S rDNA detection and CSF culture results in group I (patients group):

Parameters			16S DNA detection results		Total
			Negative N=10	Positive N=20	
CSF culture results	Negative N=25	N	10	15	25
		%	40.0%	60.0%	100%
	Positive N=5	N	0	5	5
		%	0.0%	100.0%	100%
Total N=30		N	10	20	30
		%	33.3%	66.7%	100%

Table (4): Comparison between positive and negative cases for 16s rDNA among patients with expected bacterial meningitis:

Parameters	16S rDNA detection result		P. value
	Negative (N=10)	Positive (N=20)	
Acute Onset	+ve	+ve	
CSF Aspect Turbid Hazy	 1 (10%) 9 (90%)	 20 (100%) 0 (0%)	P1 <0.001***
Total WBCs Count/Mm3 Median (Range) Mean ± SD	 585(200–3500) 784 ± 982.98	 5050(1065–38400) 7602.75 ± 8488.59	P2 <0.001***

Parameters	16S rDNA detection result		P. value
	Negative (N=10)	Positive (N=20)	
Glucose (Mg/Dl) Median (Range) Mean ± SD	 37(10–45) 34.8 ± 10.61	 19(3–30) 16 ± 6.87	P3 <0.001***
Protein (Mg/Dl) Median (Range) Mean ± SD	 80(65–300) 104.5 ± 69.58	 300(150–480) 304.35 ± 91.33	P4 <0.001***
PMNLS (%) Median (Range) Mean ± SD	 82.5(50–87) 74.4 ± 14.47	 90(85–95) 90.8 ± 2.73	P5 <0.001***
Microscopic examination • Negative • Positive	 9 (90%) 1(10%)	 13(65%) 7(35%)	P6 0.21
CSF culture • Negative • Positive	 10 (100%) 0 (0%)	 15(75%) 5(25%)	P7 0.14

Table (5): comparison between 16S rDNA detection and CSF Culture results in patients with expected VP shunt related meningitis:

Parameters			16S rDNA detection result		Total
			negative n=4	positive n=11	
CSF culture results	Negative N=12	N	4	8	12
		%	33.3%	66.7%	100%
	Positive N=3	N	0	3	3
		%	0.0%	100%	100%
Total		N	4	11	15
		%	26.7%	73.3%	100%

Table (6): Comparison between 16s rDNA gene detection and CSF Culture results in patients with expected community acquired bacterial Meningitis:

Parameters			16S DNA detection		Total
			Negative N=6	Positive N=9	
CSF culture results	Negative N=13	N	6	7	13
		%	46.2	53.8	100%
	Positive N=2	N	0	2	2
		%	0.0%	100%	100%
Total		N	6	9	15
		%	40	60	100%

Table (7): Detection of 16S rDNA in patients diagnosed by different clinical, CSF and microbiological diagnostic methods:

Categories of patients according to diagnostic method		Cases with positive 16S rDNA detection
Category A patients N=30	N %	20 66.7
Category B patients N=26	N %	20 76.9
Category C patients N=21	N %	15 71.4
Category D patients N=8	N %	7 87.5

Category A includes patients with acute onset, hazy or turbid CSF with WBCs>200/m m^3, polymorph predominance and increased protein.

Category B includes patients with acute onset, hazy or turbid CSF with WBCs >500/m m^3, polymorph predominance, increased protein and decreased glucose.

Category C includes patients with acute onset, culture negative, hazy or turbid CSF with WBCs >500/m m^3, polymorph predominance, increased protein and decreased glucose.

Category D includes patients with confirmed acute bacterial meningitis with positive CSF culture or presence of gram positive capsulated diplococci in direct film.

Table (8): Sensitivity and specificity of 16S rDNA detection based on gold standard diagnostic methods for acute bacterial meningitis:

Gold standard	16S Rdna		Total
	+ve	-ve	
+ve[1]	7	1	8
-ve[2]	0	7	7
Sensitivity	**87.5%**		
Specificity	**100%**		

[1]Patients with positive CSF culture or presence of gram positive capsulated diplococci in direct film in cases with acute onset, hazy or turbid CSF with WBCs >500/m m3, polymorph predominance, increased protein and decreased glucose levels.
[2] Control patients with expected viral meningitis

FASTA format of K. pneumoniae sub.

CGCTGCCGGCAGGCCTACACATGCAGTCG
AGCGGAAGCACAGAGAGCTTGCTCTCGGGTG
ACGAGCGGCGGACGGGTGAGTAATGTCTGGG
AAACTGCCTGATGGAGGGGGATAACTACTGG
AAACGGTAGCTAATACCGCATAACGTCGCAA
GACCAAAGTGGGGGACCTTCGGGCCTCATGCC
ATCAGATGTGCCCAGATGGGATTAGCTAGTAG
GTGGGGTAACGGCTCACCTAGGCGACGATCCC
TAGCTGGTCTGAGAGGATGACCAGCCACACTG
GAACTGAGACACGGTCCAGACTCCTACGGGA
GGCAGCAGTGGGGAATATTGCACAATGGGCG
CAAGCCTGATGCAGCCATGCCGCGTGTGTGAA
GAAGGCCTTCGGGTTGTAAAGCACTTTCAGCG
GGGAGGAAGGCGGTGAGGTTAATAACCTTGG
CGATTGACGTTACCCGCAGAAGAAGCACCGG
CTAACTCCGTGCCAGCAGCCGCGGTAATAA.

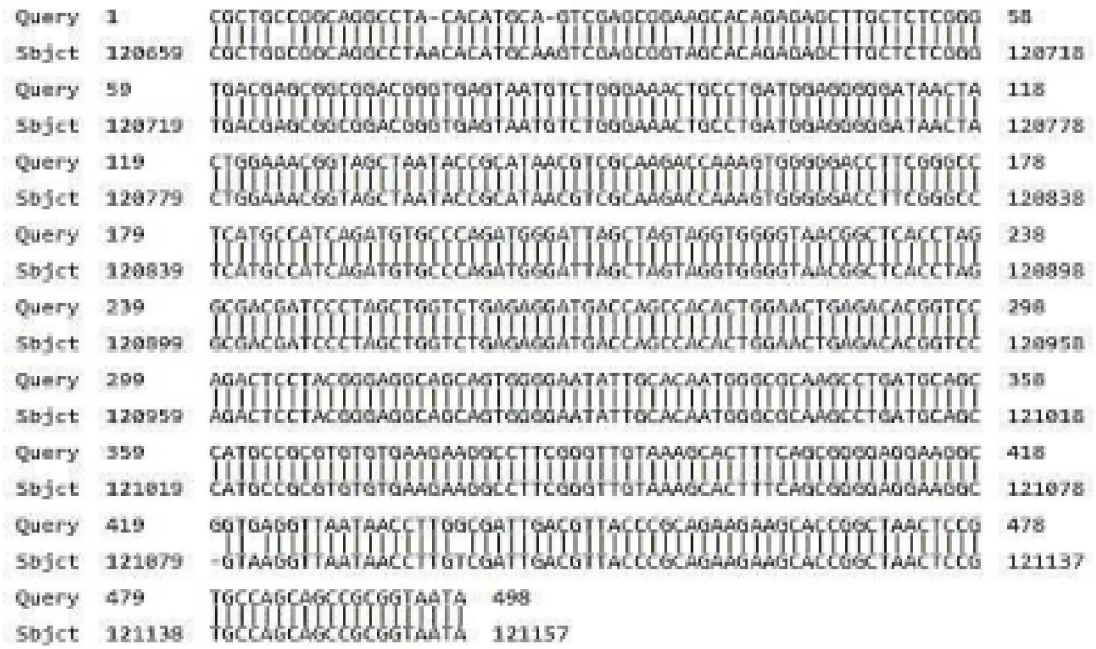

Figure (1): Gene alignment between the query and subject sequence in the gene bank database with blast identity 99% for K. pneumoniae sub. Pneumoniae

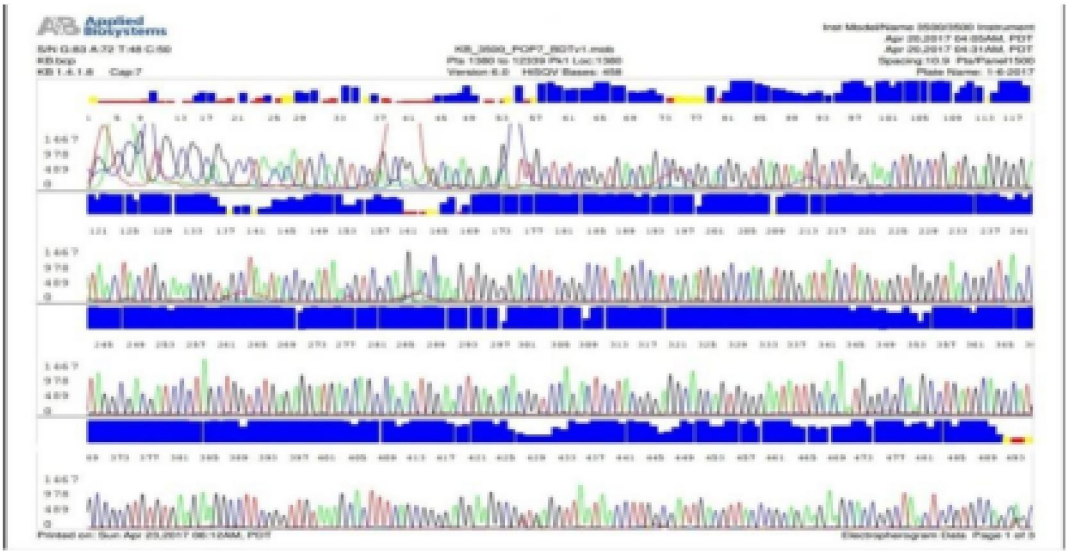

Figure (2): Electropherogram of K. pneumoniae sub. Pneumoniae

4. Discussion

In the present study the age of mean age of patients selected were 15.8 ±22.56 years in group I and 14.1 ± 21.19 years in group II. Age of the patients could greatly determine the most common bacterial species affecting them as previously discussed by **Van de Beek et al., 2016[15].**

Regarding sex, male predominance in both groups was noticed this was in coordance with. **Brouwer et al., 2010[1] and Shrestha et al.,2015[16]**, while **Dharmarajan et al.,2016[17]** demonstrated that there is no gender difference in incidence of meningitis.

In patients with nosocomial expected meningitis, they all had VP shunt as a treatment for hydrocephalus. On the other hand, in community acquired expected meningitis 9 patients had no underlying condition, 4 had a single underlying condition (e.g. diabetes, UTI, cancer and a neonate), while 2 patients had 2 underlying condition in each (diabetes & pneumonia in one patient and diabetes in cirrhotic one). Similar underlying conditions were found by other studies such as that done by **Pomar et al., 2013 [18]**. Different underlying conditions found in the present study and others could precipitate for meningitis as well as other bacterial infections, and they sometimes affect the bacterial species causing infection as well **(1).**

The CSF profile of patients expected to have acute bacterial meningitis included hazy or turbid aspect, with a total WBCs count ranging from 200 to 38400mm^3, % of PMNLs ranging from 50% to 95%, mean protein of 237.73 ± 127.08 mg/dl and mean glucose of 22.27 ±12.13 mg/dl. Patients with total WBCs count of 200/mm^3 or more were included in the present study as suggested by **Arosio et al.,2008[19]** and **Khater and Elabd, 2016[20]** while some others suggested only a count of 500/mm3 or more for suspicious of bacterial meningitis **(10,21)**

Bijlsma et al., 2016[22] demonstrated that the three parameters of CSF (WBCs, protein and glucose) are individual predictors of bacterial meningitis and at least one was present in 96% of the patients with bacterial meningitis.

On the other hand, patients expected to have viral meningitis who were used as controls had clear CSF, total WBCs count ranging from 100 to 200mm^3, % of lymphocyte ranging from 85% to 95%, mean protein of 82.14 ± 14.68 mg/dl and mean glucose of 56.43 ± 12.15 mg/dl. These characters were suggested to be associated with viral causes in patients with acute symptoms of meningitis **(23).**

Regarding direct microscopic examination of CSF for bacterial cells by Gram's stain, 8 patients out of 30 (26.7%) had positive microscopic detection of gram negative bacilli (in 2 patients), gram positive grape like cocci (in 3 patients) and capsulated gram positive diplococci (in 3 patients), this was compatable with **Arosio et al.,2008[19].**

Positive CSF culture was detected in 5 patients out of 30 (16.7%) where 2 cases had E coli and 3 had Staphylococcus (2 S. epidemidis and 1 S. aureus). **Schuurman et al., 2004[24]** found that 16% of their studied caseshad positive culture (N. meningitidis, S. pneumoniae, E coli, H. influenza and S. salvarius). As well as **Welinder-Olsson et al., 2007[25]** demonstrated that 43% had positive CSF culture (S. pneumoniae, N. meningitidis, H. influenza and S. aureus).

Negative cases of CSF culture in the present study could be explained by administration of

prophylactic antibiotics in those with VP shunt (80% were negative). Similar results were found by **Hasbun, 2016[26].**

On the other hand, negative culture in cases with expected community acquired infection (86.7%) could be explained in some cases by antibiotic initiation before sampling in whom sampling was postponed until waiting for CT result this was combatable with **Arosio et al.,2008[19]** and **Brouwer et al., 2010[1].**

Absence of bacterial growth in culture, in the present study and others, could be also attributed to presence of lower bacterial count than the sensitivity of culture, the presence of fastidious organisms or those which need special growth requirements. Moreover, some cases could have nonbacterial causes especially those with borderline characters of CSF.

In patients with community acquired infection, 2 cases had both CSF and blood culture positive. One of them had UTI where culture revealed E coli and the other had pneumonia where the culture revealed S aureus. These cases suggested distant spread of infection from infected focus to the meninges as described by **Brouwer et al.,2010[1]** and **Pomar et al.,2013[18]**.

In the present study, it was found that 16S rDNA was detected in (66.7%) of cases expected to have acute bacterial meningitis while not in any cases expected to have viral infection (controls), which indicate a specificity of 100% to bacterial infection. Many other studies demonstrated that 16S rDNA is present only in bacterial genome **(27).**

However, exclusion of bacterial contamination should be done by using complete aseptic precaution during sampling, this was recommended by several studies (**24,28).**

Patients with VP shunt revealed 16S rDNA in CSF of 73.3% of cases. The 4 negative could have low bacterial count beyond the test sensitivity,. In a similar study, **Banks et al.,2005[29]** demonstrated that 70% of their studied cases were positive for 16S rDNA.

On the other hand, patients with expected community acquired infection revealed positive 16S rDNA in CSF of 60% of them. Other studies included that of **Arosio et al.,2008[19]** who found that 16S rDNA was positive in 75% and **Rafi et al.,2010[30]** who found that 16S rDNA was positive in 80% of cases. This difference in the result could be attributed to the different selection of cases, where some of them could have other causes for meningitis, or early viral meningitis which could be mistaken with bacterial one.

Regarding detection of 16S rDNA in culture positive and negativecases, it was found that all culture positive cases had 16S rDNA while 60% had 16S rDNA in culture negative cases. These included 66.7% in cases with shunt and 53.8% in cases with community acquired infection. Similar studies revealed that 78.9% and 75% of cases with negative culture were positive for 16S rDNArespectively (**25,19**). It was suggested that 16S rDNA detection has the ability to identify even dead bacteria due to antibiotic therapy and rare, uncommon, fastidious and anaerobic bacteria **(19,24 and 31).**

On comparing patients with positive and negative 16S rDNA, it was noticed that there were more turbidity in CSF, more protein increase and more glucose decrease together with more detection of bacteria in direct microscope or culture in those with positive 16S rDNA than negative ones. This could be explained by presence of some cases with low bacterial count in CSF within patients who revealed negative both 16S rDNA and culture.

The sequencing of 16S rDNA in patients with VP shunt revealed the following bacterial species E coli (3cases), K. pneumoniae (3cases), S.epidermidis (3cases) and P. aeruginosa (2 cases). This was in similarity with **Sarguna and Lakshm,2006[32]** and **Lee et al.,2012[33].** However **Bokhary and Kamal, 2008[34]** demonstrated that GNB represented 81.3% of pathogens and gram positive bacteria (all were S. epidermidis) were isolated in 12.5% only.

The difference in bacterial species found in different studies could be attributed to different selection of patients, the difference in antibiotic policy and the most predominant infection in the hospital.

Bacterial species detected by 16S rDNA sequencing in community acquired cases revealed E coli, S. pneumoniae, S. aureusand K. pneumoniae sub. Pneumoniae this was compatable with results of some studies as **Arosio et al., 2008[19].** Also**, Khater and Elabd, 2016[20]**. Furthermore, **Pomar et al.,2013[18]** found that gram negative bacilli were a significant cause of bacterial meningitis and could be the third or fourth cause of nontraumatic bacterial meningitis after S pneumoniae and N. meningitides.

On correlating the detected bacterial species with age in community acquired cases, it was found that the causative organism in neonate was E coli, in infants & children was S. pneumoniae and in adolescent & adults were E coli, S. pneumoniae, S. aureus and K. pnumoniae sub. Pneumoniae. This agreed with those who classified causative agents of meningitis according to age **(1,15)**.

Some other studies found that Gram-negative bacilli especially E. coli are emerging as an important cause of community acquired acute bacterial meningitis in adults **(35)**. Also, **Aguilar et al., 2010[36]** found that staphylococcal infection is associated with elders while **Gituro et al.,2017[37]** demonstrated that S. pneumonia was the most common cause of bacterial meningitis in infants and children (55%) followed by N. meningitides (32%) and H. influenza (14%).

In the present study the correlation between the detected bacterial species and the underlying condition, could be explained by that certain underlying condition such as diabetes, cancer, presence of infective focus, immunosuppression or other debilitating diseases could precipitate for infection with certain bacterial species that can be spread from other infected focus **(1,18).**

In the present study, it was found that detection rate for 16S rDNA increased when patients were selected based on combined diagnostic criteria including acute clinical manifestation, CSF characters and microbiological findings. These findings indicated that detection of 16S rDNA could greatly help in diagnosis in many cases of culture negative bacterial infections as well as in excluding borderline cases mistaken with bacterial infection. This was also demonstrated by other studies such as that done by **Rafi et al.,2010[30]** and **Rampini et al.,2011 [38]**.

In this study, the sensitivity of 16S rDNA detection and sequencing was estimated in 8 patients who had confirmed acute bacterial meningitis based on gold standard diagnosis, with a sensitivity of 87.5%.

Other studies revealed that the sensitivity was 59%, 75% and 90% as demonstrated by **Welinder-Olsson et al.,2007[25], Arosio et al.,2008[19] and Khater and Ehab,2016[20]** respectively. Difference in sensitivities between different studies could be attributed to different selection of patients.

Conclusion

Direct detection and sequencing of the 1st 500 bp of 16S rDNA by using cycle sequencing can provide a specific, sensitive, early, rapid and non-expensive method for improving the microbiological diagnosis of acute bacterial meningitis, especially after initiation of antimicrobial therapy and in culture negative cases. In spite of lacking bacterial isolation and sensitivity test, it could re-optimize the empirical use of antibiotics based on the identified bacterial species.

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