Syndromic Evaluations of CNS Infections by Multiplexed PCR Panels Enable Rapid Detection and Treatment of Infections

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Abstract: *A variety of pathogens can cause infections in the central nervous system (CNS) in children with devastating consequences. Diagnostic methods that rapidly and comprehensively assess the causative agent facilitate early detection and treatment, and could alter the course of illness avoiding long-term brain injury. Here, we examine the infectious causes of CNS infections in children using multiplex PCR to detect a comprehensive panel of pathogens known to cause such infections*

Keywords: CNS infections, Pediatric, Multiplex PCR, Molecular diagnostics

1. Introduction

Infection of the central nervous system is a life-threatening condition in the pediatric population [1]. The clinical manifestations in children are also quite different and variable compared with adults. CNS infection can present as meningitis, encephalitis, myelitis or abscess. They are most commonly spread hematogenously but can also spread from adjacent structures (otitis, sinusitis, dental abscess) or through neural pathways [4]. They can also be a result of invasive procedures (e.g. neurosurgery) and noninvasive trauma with skull fractures or due to the presence of a foreign body (e.g. ventricular shunt). Infections can also be contracted due to immune compromised condition of the patient. The blood brain barrier (BBB) prevents the entry of pathogens and inflammatory cells, providing effective protection against infections [2]. However, once the BBB is breached, these infections are difficult to treat because of reduced permeability of antimicrobials as well. This study to assess the comprehensive CNS Panel designed by iGenetic Diagnostics, helps to identify pathogens within 24 hours.

2. Literature survey

It is very challenging to determine the likely causative organism in CNS infections and start empirical treatment due to nonspecific clinical features [3]. CNS infections can present as acute or chronic infections and can lead to systemic infections. Acute meningitis is seen in bacterial and viral infections with acute onset of fever, headache, vomiting, meningismus, altered mental status; rapid progression over hours to days. Acute bacterial meningitis remains a major cause of mortality and long-term neurological disability. Post infectious syndromes are associated with viruses/vaccines; symptoms are varied depending on the lesions: acute disseminated encephalomyelitis, transverse myelitis, optic neuritis, multiple sclerosis.

There are several factors that can influence the type of organism infecting the CNS. Some of these are age, immune status, epidemiological trends and systemic infections. Also, a wide range of different organisms like bacteria, viruses, fungi, protozoa, or parasites can cause these CNS infections. This results in treatment with broad spectrum antimicrobials resulting in increased antimicrobial resistance, failure of treatment, serious side-effects and increased treatment costs.

3. Problem Definition

This study was conducted to assess the effectiveness of comprehensive CNS panel designed by iGenetic Diagnostics in detecting the most common organisms known to cause CNS infections. This panel which included bacterial, viral, fungal, Mycobacterial and parasitic pathogens (Table 1) was used to screen samples of pediatric patients suspected of CNS infections.

Table 1: iGenetic Extended Comprehensive CNS Panel

Gram Positive Bacteria	Gram Negative Bacteria	Viruses
Staphylococcus aureus	Rickettsia species	Epstein Barr Virus
Group B Streptococcus	**Atypical Bacteria**	Adenovirus
Streptococcus pneumoniae	Mycoplasma pneumonia	Parechovirus
Enterococcus species	**Acid fast bacilli**	Measles Virus
Listeria species	Mycobacterium Tuberculosis Complex (MTC)	Rubella Virus
Gram Negative Bacteria	Non-Tuberculous Mycobacteria (NTM)	Dengue Virus
Klebsiella pneumoniae	**Viruses**	West Nile Virus
Escherichia coli	Herpes Simplex Virus 1&2	Rabies Virus
Enterobacter aerogenes	Cytomegalovirus	Nipah Virus
Pseudomonas aeruginosa	Varicella Zoster Virus	Chikungunya Virus
Acinetobacter baumanii	Human Herpes Virus 6	Chandipura Virus
Hemophilus influenzae B	John Cunningham Virus	**Fungi**
Neisseria meningitidis	Enterovirus	Candida species
Bacteroides fragilis	Japanese Encephalitis Virus	Aspergillus species
Salmonella species	Mumps Virus	Cryptococcus neoformans
Leptospira species		**Parasite**
Treponema pallidum		Toxoplasma gondii

4. Method/ Approach

Cerebrospinal fluid (CSF) samples were obtained from 49 pediatric cases with suspected CNS infections. All samples collected between January 2017 and June 2017 and processed at iGenetic Diagnostics were included in the study. The CSF was spun down and 800ul of the supernatant along with the pellet (if any) was used for DNA and RNA extraction (400ul each). DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Germany) while RNA was extracted using QIAamp Viral RNA Mini Kit (Qiagen, Germany). cDNA was made from RNA using the SensiFAST™ cDNA Synthesis Kit (Bioline, UK).

The DNA and cDNA were then used for diagnosing infections using the iGenetic Extended Comprehensive CNS panel. The composition of this multiplexed PCR panel is shown in table 1. In all, 20 viruses, 18 bacteria, 3 fungi, 1 parasite, Mycobacterium tuberculosis complex (MTC) and Non-Tuberculous Mycobacteria (NTM) were examined. The ordering physicians sometimes chose to customize the panel based on clinical history.

5. Result / Discussion

5.1 Result

Multiplexed PCR assays, in the form of a comprehensive panel, were run for 45 pathogens, (microbial, viral, fungal, Mycobacterial and parasitic) on 49 pediatric CSF samples. Each sample was screened for a variety of pathogens in a parallel multiplex mode to rapidly identify the cause of infection. Few observations were made based on the outcome of this study

Bacteria and viruses cause around 70 % of the pediatric CNS disease
Of the 49 CSF samples analyzed, 23 cases were positive. Viral infections accounted for nearly 39.1% (n=9) whereas bacterial infections accounted for 30.4% (n=7) of the cases. About 21.70% (n=5) of the cases reported positive for, fungi and 30.40% (n=7) for Mycobacteria (Figure 1)*.
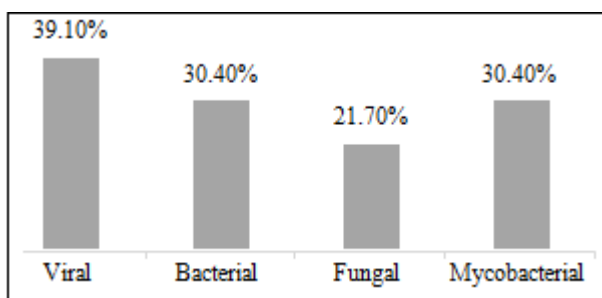

Figure 1: Causative etiology in pediatric CNS infections (n=23)
*% may exceed more than 100 as cases had more than one infections

Treatment for each kind of pathogen is largely different and hence this data could prove useful in deciding the appropriate treatment. In addition, several polymicrobial infections were also detected.

Enterovirus cause over half of all viral CNS infections in children:
The iGenetic Extended Comprehensive CNS panel was used to screen for 20 viruses. The incidence of various viruses is shown in Figure 3. Enterovirus was found to be the most common pathogen causing CNS infections in children and accounted for 55.56% of viral CNS infections. It was detected mostly as a single infection but was sometimes observed as a mixed infection with other pathogens. Epstein–Barr viruses (22.22%) were the second most common type of viruses detected in pediatric CNS samples
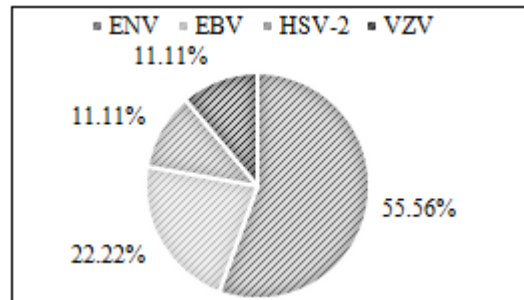

Figure 2: Prevalence of viral pathogens in 49 pediatric CSF samples (n=9)

Occurrence of bacterial pathogens in pediatric CNS disease
Of the 23 positive cases, 7 samples tested positive for bacterial infections. Both Gram positive and Gram negative bacteria were found in these infections. However, the prevalence of Mixed Gram positive and Gram negative infections were seen to be higher (See figure 3).
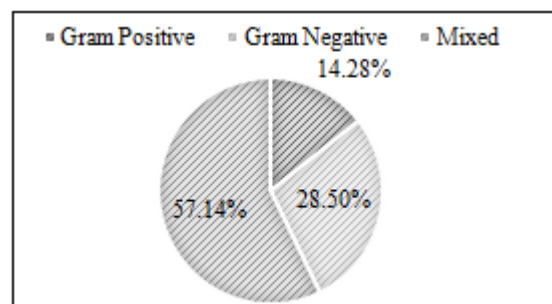

Figure 3: Occurrence of Gram positive and Gram negative bacterial pediatric CNS infections (n=7)

The distribution of bacteria found in pediatric CNS infections is presented in Table 2

Streptococcus spp was found in 80% of all infections caused by Gram positive bacteria. Among Gram negative bacteria, the Enterobacteriaceae family was responsible for 86% of Gram negative pediatric CNS infections.

Table 2: Bacteria causing pediatric CNS infections

Bacteria	Detected in (No. of cases)	Percentage
H. influenzae	1	14.2%
S. pneumoniae	2	28.4%
E. coli	3	42.8%
K. pneumoniae	2	28.4%
Enterococcus spp	1	14.2%
E.aerogenes	1	14.2%
Streptococcus spp	2	28.4%
Staphylococcus spp	1	14.2%

*% may exceed more than 100 as cases had more than one type of bacteria

Fungal CNS infections in children
21.7% of pediatric CNS infections are caused by fungi. Both Aspergillus spp and Candida spp were found to cause fungal CNS infections in children. Aspergillus spp accounted for over 80% of these infections (Figure 4)*.
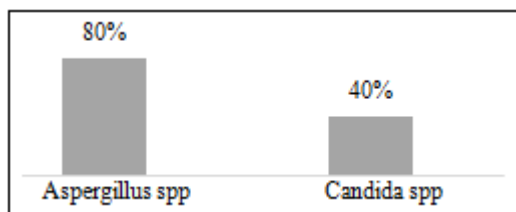

Figure 4: Occurrence of fungal pathogens in pediatric CNS infections (n=5)
*% may exceed more than 100 as cases had more than one type of fungal infections

CNS infections caused by Mycobacteria in children
MTC and NTM were responsible for 30.4% of all pediatric CNS infections. Of these, NTM cause higher of Mycobacterial CNS infections than MTC in children (Figure 5).
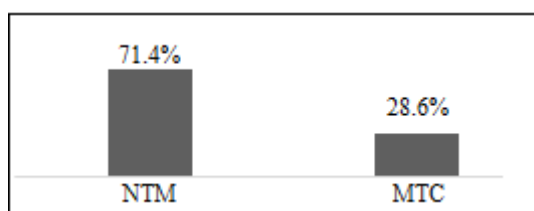

Figure 5: Occurrence of Mycobacterial pathogens in pediatric CNS infections (n=7)

5.2. Discussion

Pathogen detection by culturing the causative organism is the gold standard for diagnosis of infections. This can easily take around 72 hours for bacteria and longer for fungi. Besides, microbiological methods show false negativity in patients pre-treated with antimicrobials, and have low sensitivity for slow growing, intracellular and fastidious microbes. For example, Mycobacterial infections may take weeks to detect. Also, routine diagnostic labs cannot culture viruses which are known to cause a significant proportion of CNS infections. Detection of viral infections is based on serological tests that suffer from low sensitivity and specificity. Molecular methods for pathogen identification can overcome these challenges [3] and could result in a better outcome due to timely diagnosis and evidence-based use of antibiotics.

In the current study, we found that 70% of pediatric CNS infections are caused by bacteria and viruses. Bacteria and viruses were almost equally represented. Viruses were actually found in a slightly higher proportion than Bacteria. Which can further be tested with higher sample size.

Bacterial meningitis has a high fatality rate and hence requires rapid diagnosis and appropriate treatment [8]. Among those that survive bacterial meningitis, a significant number of patients suffer from disabling neuropsychological deficits [9] with children showing difficulties in learning, impaired short-term memory, behavioral deficits and poor academic performance [10]. In the current study, Streptococci were found to be the most common Gram positive organism among bacterial CNS infections. Even among these, S. pneumoniae was the highest. This is easily preventable by vaccinations thus making a case for increasing awareness about pneumococcal vaccinations in India.

Surprisingly, we found a higher incidence of Gram negative organisms in bacterial CNS infections in children. Although most reports from the West primarily discuss pneumococcal or meningococcal bacterial infections in children, we found that Enterobacteriaceae family members are the most commonly encountered Gram negative bacteria in bacterial CNS infections. A majority of viral CNS infections are caused by Enteroviruses. In our study, more than 55% of cases with viral CNS infections were enteroviral. Our data is supported by other studies showing coinfections of bacteria with Enteroviruses [6, 7].

Herpes viruses were seen in 11.11% of viral CNS infections in children. Fungal CNS infections can be caused by a wide variety of fungi and can be devastating [11]. Despite the aggressive nature of these infections, the clinical presentation may be subtle, nonspecific and difficult to diagnose. In this study, we focused on detecting the most common causes of fungal CNS infections, namely, Aspergillus spp, Candida spp and Cryptococcus neoformans. We reported fungal CNS infections in 21.7% of pediatric cases and identified the causative agent as Aspergillus spp and Candida spp with 80% of the cases caused by the former.

One percent of TB cases manifest as CNS infections [12]. These occur predominantly in HIV infected individuals and children [13]. In this study, we found that 30.4% of all CNS infections were caused by Mycobacteria including MTC and NTM.

6. Conclusion

The iGenetic Extended Comprehensive CNS panel employs a multiplexed PCR assay that screens simultaneously for a wide variety of pathogens giving a higher detection rate as compared to point-of-care devices like Biofire [14,15]. The comprehensive iGenetic panel facilitated detection of a wide variety of CNS pathogens within 8 hours that enables initiation of appropriate treatment, however the results can be validated with higher sample size in further phase of the study.

In conclusion, comprehensive molecular diagnostic panels enable accurate diagnosis, timely treatment decisions and better outcome for patients with CNS infections..

7. Future Scope

Further phase of the study will be conducted with higher sample size to assess the results among different age group of children and with different age groups and different

demographic variability

References

[1] Sahu R. N., R. Kumar, and A. K. Mahapatra. 2009. Central nervous system infection in the pediatric population. J Pediatr Neurosci. 4(1):20-4.

[2] Chaudhuri J. D. 2000. Blood brain barrier and infection. Med Sci Monit. 6(6):1213-22.

[3] He T., S. Kaplan, M. Kamboj, Y.W. Tang. 2016. Laboratory Diagnosis of Central Nervous System Infection. Curr Infect Dis Rep. 18(11):35.

[4] Swanson P. A. 2nd, and D. B. McGavern. 2015. Viral diseases of the central nervous system. Curr Opin Virol. 11:44-54.

[5] Rotbart H. A. 2000. Viral meningitis. Semin Neurol. 20(3):277-92.

[6] Basmaci R., P. Mariani, G. Delacroix, S. Azib, A. Faye, M. K. Taha, E. Bingen , S. Bonacorsi ,J. R. Romero , H. A. Rotbart ,A. C. Nyquist, and F. S. Nolte. 2011. Enteroviral meningitis does not exclude concurrent bacterial meningitis. J Clin Microbiol. 49(9):3442-3.

[7] Calvo C., P. Gallardo, P. Torija, S. Bellón, A. Méndez-Echeverría, T. Del Rosal , F. Baquero-Artigao, T. Sainz, M. Romero, M. Cabrerizo. 2016. Enterovirus neurological disease and bacterial coinfection in very young infants with fever. J Clin Virol. 85:37-39.

[8] Hoffman O. and R. Joerg. Weber. 2009. Pathophysiology and treatment of bacterial meningitis. Ther Adv Neurol Disord. 2(6) 401-412

[9] Merkelbach S., H. Sittinger, I. Schweizer, and M. Müller. 2000. Cognitive outcome after bacterial meningitis. Acta Neurol Scand. 102(2):118-23.

[10] Grimwood K., P. Anderson, V. Anderson, L. Tan, and T. Nolan. 2000. Twelve year outcomes following bacterial meningitis: further evidence for persisting effects. Arch Dis Child. 83(2): 111–116

[11] McCarthy M. W., D. Kalasauskas, V. Petraitis, R. Petraitiene, T. J. Walsh. 2017. Fungal Infections of the Central Nervous System in Children. J Pediatric Infect Dis Soc. 6(3):e123-e13

[12] Ingole R., Garg R. K., Malhotra H. S., Jain A., Kumar N., Rizvi I., and Garg R. Spectrum of central nervous system tuberculosis: An experience from a large tertiary care institution of India. Ind J. Tuberculosis. https://doi.org/10.1016/j.ijtb.2017.05.011

[13] Chatterjee S. 2011. Brain tuberculomas, tubercular meningitis, and post-tubercular hydrocephalus in children. J Pediatr Neurosci. 6(Suppl 1):S96-S100

[14] Flor A., J. A. Capdevila, N. Martin, J. Gavaldà, and A. Pahissa. 1996. Nontuberculous mycobacterial meningitis: report of two cases and review. Clin Infect Dis. 23(6):1266-73.

[15] Tarai B. and P. Das. 2018. FilmArray® meningitis/encephalitis (ME) panel, a rapid molecular platform for diagnosis of CNS infections in a tertiary care hospital in North India: one-and-half-year review. Neurol Sci. Sep 25. doi: 10.1007/s10072-018-3584-y. [Epub ahead of print]

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