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Challenges in diagnosis of central nervous system infections using conventional method: Need for better approach in Rwanda

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Central nervous system (CNS) infection is a common and serious disease that needs rapid and appropriate diagnosis for an appropriate treatment. However, in most recourse limited setting including Rwanda, conventional microbiological method is the only way to establish a confirmed infectious etiology. This was a 4 years retrospective review of registers and electronic laboratory records aimed to determine the causative agents in hospitalized patient's suspected to be suffering from CNS infection at four referral hospitals in Rwanda. In this review, the majority of participants (48%) were in the age group between 25 and 44 years (median = 34), with 53 and 47% being males and female, respectively. Cerebrospinal fluid (CSF) was clear in 67% (112/168), turbid in 30% (50/168) and bloody in 3% (6/168) patients. Only 1% (2/168) of the samples had WBC count >1,000 cells/mm^3 and 13% (21/168) had WBC count between 101 and 1,000 cells/mm^3, WBC count between 10 and 100 cells/mm^3 was present in 10% (17/168) whereas <10 cells/mm^3 was present in 76% of the samples. The present data above was from one selected referral hospital out of four sites used in this study, whereby all required data were funded during data collection. Out of 208, positive CSF was identified from four sites; *C. neoformans* was the most frequent pathogen isolated, followed by *Streptococcus pneumoniae* representing 71.6 and 9.6%, respectively. Other pathogens identified included *Acinetobacter* spp. which represented 4.3%, *S. aureus* 3.8%, *E. coli* 2.8% and *K. pneumonia* 1.9%. Both *N. meningitides* and *H. influenzae* type B were isolated in only 0.48% for each. The present study reveals that the diagnostic of CSF infection using conventional method is alarmingly low across all tertiary hospitals, suggesting further studies using molecular methods to shed light on the etiological agent of CNS infections in Rwanda.

Key words: Central nervous system (CNS) infection, conventional method, causative agents, diagnostic capacity and resource poor setting.

INTRODUCTION

Meningoencephalitis is a disease characterized by, inflammation of the meninges and brain tissue due to infection by microorganisms such as bacteria, virus, parasites and fungi (Shaban and Siam, 2009). In the clinical setting, this diagnosis is often considered in any patient presenting with fever, headache and/ or altered mental status who happens to be found with meningeal irritation signs on physical examination (Fouad et al., 2014).

In infected individuals with encephalitis, symptoms such as headache, fever, vomiting, confusion and light-sensitiveness are common and can in severe cases also cause unconsciousness, seizures and paralysis. Viral meningoencephalitis is the most common but least severe form with almost all patients recovering without any permanent pathological changes, although full recovery might sometimes take weeks.

Bacterial meningoencephalitis is often more severe than viral meningoencephalitis and can lead to permanent pathological changes or death in around 50% of untreated cases, and accounts for around 170,000 deaths globally each year (Boving et al., 2009). Most cases of bacterial meningoencephalitis are caused by *Streptococcus pneumonia* (Afifi et al., 2007). *Streptococcus agalactiae, Neisseria meningitidis, Listeria monocytogenes* and *Hemophilus influenza B* are examples of bacterial meningoencephalitis (Akhvlediani et al., 2014). Bacterial meningitis reaches the sub-arachnoid space by hematogenous route or may directly reach the meninges in patients with parameningeal focus of infection. Bacterial meningitis usually can cause brain damage, hearing loss, limb amputation, learning disabilities and even death (Baskin and Hedlund, 2007; Minjolle et al., 2002; Růzek et al., 2007).

Fungi cause severe infections but are much less frequent than bacterial or viral infections (Baskin and Hedlund, 2007; Minjolle et al., 2002; Růzek et al., 2007). The most common causes of fungal meningoencephalitis are *Cryptococcus neoformans, Candida albicans* and *Aspergillus* species mainly in immune-compromised patients (Baskin and Hedlund, 2007). *C. neoformans* is an encapsulated basiodiomycetes fungus of medical importance, capable of crossing the blood brain barrier and causing meningitis in both immunocompetent and immunocompromised individuals.

The best characterized virulence factors for *C. neoformans* include the production of polysaccharide capsule, the formation of melanin and the ability to grow at 37°C which is an essential virulence factor for pathogenesis. Polysaccharide productions within phagocytic cells contribute to fungal survival (Steen et al., 2003). The main symptoms of fungal meningoencephalitis are fever, vomiting, headache, stiff neck, sensitivity to light and drowsiness. The serious and disabling complications include hearing loss, brain damage or learning difficulties (Baskin and Hedlund, 2007; Minjolle et al., 2002; Růzek et al., 2007).

In order to minimize unnecessary antibiotics, antiviral and antifungal prescriptions and to determine the appropriate treatment, it is important to identify at an early stage whether meningitis, encephalitis or meningoencephalitis is caused by bacteria, viral or fungal pathogens (Baskin and Hedlund, 2007). This also would minimize the exposure of patients to side effects of medications they do not require. The current treatment guidelines for the treatment of meningoencephalitis in developing countries are based on data obtained from countries with robust economies and strong immunization programs.

This might be misleading in resource-limited settings, which may have completely different disease aetiologies. Thus, there is a need for descriptive epidemiology of infective agents of meningoencephalitis in different geographical locations, as the pathogens vary depends on environmental circumstances, amongst other things.

Currently in Rwanda, conformation of infectious aetiology in patients with acute Central nervous system (CNS) infections completely relies on conventional diagnostic methods, consisting of routine cultures. The availability of reliable and improved diagnostic tools such as molecular method is extremely limited. The current study was undertaken to examine the utility of conventional microbiological methods in the identification of the causative agents in hospitalized patients, suspected of cerebrospinal fluid (CSF) infection. It also aimed to provide useful baseline data, highlighting the major gap in the diagnostic capacity for CNS infection in Rwanda.

MATERIALS AND METHODS

All patients meeting the inclusion criteria and willing to participate in

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the study as indicated by signing an informed consent were included in the study. For each patient, laboratory registers were accessed to extract information relevant to this study. This consisted of descriptive retrospective hospital record results from analyzed CSF samples. Using conventional microbiological methods, the present hospital based study findings were drawn from 2,410 hospitalized patients with suspected CNS infections admitted in 4 referral hospitals in Rwanda.

Complimentary data was obtained using electronic laboratory records by, retrieving retrospectively hospital record results from analyzed CSF samples. All data was captured from analyzed CSF samples results as evidence of meningoencephalitis or absence of causative agents. The data was recorded in Excel spreadsheets and was transferred to SPSS for further analysis.

CSF samples collection and transport

Prior to data collection, the principal investigator held a meeting with all the experienced staff in cerebrospinal fluid (CSF) collection in all the research sites, to discuss sample collection and transportation to a central laboratory. The CSF samples were obtained using standard guidelines for sterile clinical procedures. In brief, before lumbar puncture procedure, the patients were made to lie down at an appropriate position, the skin was disinfected along a line drawn between the crests of the two ilia with 70% alcohol and iodine and allowed to dry completely.

The clinician then injected the spinal needle into the skin between the 4 and 5th lumbar vertebral spines with the bevel of the needle facing up. As soon as the needle was in position, the CSF pressure was measured and a sample of 3 to 4 ml of the fluid was collected in two sterile screw tubes for testing, using conventional and molecular diagnostic methods.

Collection specimen

At all sites, the obtained CSF were collected prior to antimicrobial therapy and placed into at least 3 separate sterile leak proof tubes before being transported to the laboratory. One tube from the collected CSF specimens was transported to bacteriology laboratory immediately without refrigeration. The CSF was processed in a biological safety cabinet to avoid contamination of the specimen and/or the primary inoculation medium. Blood agar plate, chocolate agar, thioglycolate broth and Sabouraud agar were used for the culture of specimens.

Processing of CSF and gram staining

Initial processing of CSF started by recording the volume of CSF and its gross appearances namely clear, bloody, cloudy, or xanthochromic. Clear CSF samples were tested using cryptococcal latex agglutination and India ink microscopy for detection of cryptococcal neoformans antigen and yeast cells surrounded by a characteristic polysaccharide capsule, respectively. Adequate turbid samples were tested using latex agglutination for detection of specific polysaccharide surface antigens, for most common bacteria as causative agents of meningitis. The specimen was then centrifuged for 20 min at 1,500 to 3,000 ×*g* if the volume recorded was >1 ml. The sediment was vortexed vigorously for at least 30 s to resuspend the pellet.

Using a sterile pipette, media was inoculated by placing 1 or 2 drops of sediment on an alcohol-rinsed slide, allowing drop to form a large heap. The slide was then air dried on a slide warmer before being gram stained as described by Fouad et al. (2014). The CSF gram stained smears were examined and interpreted immediately and all positive smears were immediately reported to the physician and nursing unit by telephone. The telephone notification was documented.

Culture examination

All collected CSF samples in four referral hospitals were transported within 1 h and centrifuged at 1000 ×*g* for 10 to 15 min with supernatant, used for rapid diagnostic test. Sediment was used for gram stain and primary plating on chocolate, blood agar, MacConkey agar and sabouraud dextrose agar.

All plated and thioglycolate broth media were examined for macroscopic evidence of growth. With no visible growth on the culture media, broth was re-incubated and negative plates were examined daily for 72 h before discarding. Broth media was also examined daily for 5 to 7 days before discarding.

Culture with growth and organism identification

From colony appearance, colony was picked to prepare gram stain broth, if positive. Semi quantitative growth was put on plated media and gram stain prepared for each morphotype. The microorganisms were identified based on the morphology of colonies, and by biochemical reaction and serotyping.

Antimicrobial sensitivity was also done according to the isolated microorganisms. The physician was notified of culture findings and antibiotic sensitivity patterns.

Ethical considerations

Before any data collection, ethical approval to conduct the study was obtained from Rwanda National Ethical Committee (No.472/RNEC/2009) and respective hospital ethical committees. Privacy and confidentiality of the patients were upheld at all times. There were no personal identifiers in all the samples obtained and instead, a unique number was used for each sample.

RESULTS

As shown in Table 1, the majority of participants (48%) were in the age group between 25 and 44 years (median = 34), with 53 and 47% being males and female, respectively. Macroscopic appearances and cytological (cell count) findings of CSF samples in patients with clinically diagnosed central nervous system infections were available and complete at one site of the study as described in Tables 2 and 3, respectively. CSF was clear in 67% (112/168) patients and turbid in 30% (50/168) patients. About one-quarter, that is 3% (6/168) patients had their CSF contaminated with blood.

Table 1. Sample descriptions of the study by age group and gender.

Age group (median: 34 years)	Gender		Total (%)
	F	M	
<5	4	9	13 (8)
5-14	8	8	16 (10)
15-24	12	3	15 (9)
25-34	25	19	44 (26)
35-44	11	25	36 (21)
45-54	11	17	28 (17)
>55	8	8	16 (10)
Total	79	89	168 (100)

Table 2. Macroscopic appearance of CSF samples in CSF culture in patients with clinically diagnosed central nervous system infections.

CSF appearance	CSF culture	
	Frequency	Percent
Clear	112	67
Turbid	50	30
Bloody	6	3
Total	168	100

Table 3. CSF WBC count distribution according to CSF culture in patients with clinically diagnosed CNS infections.

WBC count in CSF	CSF culture	
	Frequency	Percent
<10	128	76
10-100	17	10
101-1000	21	13
>1000	2	1
Total	168	100

There was no difference in culture positivity rate (33 vs. 28%) in patients with turbid CSF compared to patients with clear CSF. Blood stained CSF had a culture yield of 17%. CSF cytology revealed that only 1% (2/168) of the samples had WBC count >1,000 cells/mm^3, 13% (21/168) had WBC count between 101 to 1,000 cells/mm^3, WBC count between 10 and 100 cells/mm^3 was present in 10% (17/168) whereas <10 cells/mm^3 was present in 76% of the samples. The presented data above was from one selected referral hospital out of four sites used in this study whereby all required data were funded during data collection.

The patients with elevated CSF (>1,000 WBC/mm^3) WBC count had a culture positivity rate of 55.5%. Distribution of laboratory culture findings is shown in Table 4. *C. neoformans* was the most frequent pathogen isolated followed by *S. pneumonia,* representing 71.6 and 9.6% of isolates, respectively. Other pathogens isolated included *Acinetobacter* spp (4.3%), *S. aureus* (3.8%), E*. coli* (2.8%) and *K. pneumonia* (1.9%). Both *N. meningitides* and *H. influenzae* type B were isolated in 0.5% each.

Table 4. Distribution of laboratory culture results of 208 positive CSF among 2,410 hospitalized patients with clinically diagnosed CNS infections.

Pathogen	2009		2010		2011		2012		Total
Pathogens isolated fungi	**Frequency**	**Percent (%)**	**Frequency**	**Percent (%)**	**Frequency**	**Percent (%)**	**Frequency**	**Percent (%)**	**Frequency**
C. neoformans	34	67	41	73	36	69	38	78	149
Pathogens isolated bacteria									
S. pneumoniae	7	14	5	9	2	4	6	12	20
N. meningitidis	0	0	0	0	1	2	0	0	1
H. influenzae type B	0	0	0	0	1	2	0	0	1
S. aureus	4	8	1	2	3	6	0	0	8
Acinetobacter spp.	3	6	1	2	3	6	2	4	9
E. coli	0	0	2	4	2	4	2	4	6
K. pneumoniae	0	0	0	0	3	6	1	2	4
Others*	**3**	**6**	**6**	**11**	**1**	**2**	**0**	**0**	**10**
Total	**51**	**-**	**56**	**-**	**52**	**-**	**49**	**-**	**208**

DISCUSSION

Central nervous system (CNS) infection is a common and serious disease that needs rapid and appropriate diagnosis for an accurate treatment. The availability of reliable and improved diagnostic tools such as molecular method is extremely limited in resource-constrained countries such as Rwanda, and therefore conventional microbiological method is the only way to establish a confirmed infectious etiology in patients with acute CNS infections.

In the present study, demographic data review showed that majority of participants (48%) were in the group age between 25 and 44 years (mean = 34), with 53% being males; these findings are consistent with those of Fouad et al. (2014), who confirmed that males were more significantly affected with bacterial meningitis than females; 61 versus 39%, respectively. The majority of the patients in the present study were middle aged as has previously been described. A study by Fouad et al. (2014) found that patients with meningoencephalitis were middle aged with a mean age of 38.3 year. The reason for this age group being most affected is not known.

The low yield of microorganisms from CSF demonstrated in this 4-year retrospective review is similar to that reported in earlier studies (Becerra et al., 2013). For instance, our finding of 8.6 bacterial culture yield is comparable with studies in developing countries which reported low rates of culture positive CSF samples ranging from 8 to 10% (Wu et al., 2013) but higher than a study finding in Georgia (Khater and Elabd, 2016) which reported a positivity rate of 3.6%. There are several reasons why the diagnostic yield may be low when conventional microbiological methods are employed to identify etiological causes of CNS infections.

A potential reason for these results may be the known poor performance of conventional microbiological methods in the etiological confirmation of causative microorganism in CNS infections. Other possible reasons reported elsewhere indicate that, low diagnostic yield of conventional microbiological methods may be largely affected by the antibiotic use prior to

hospital admission. In fact, a culture positivity rate of 10% has been reported in patients previously treated with antibiotics in developing countries (Wu et al., 2013). Although information on prior antibiotic use was not recorded, previous use of antibiotics may have been higher in our study population, because the majority of these patients had been treated in lower level health facilities before being referred and admitted to a referral health facility.

Additionally, the observed low diagnostic yield could also suggest that other causative agents whose identification requires more improved methods are not used in this group of patients such as *Mycobacterium tuberculosis* or viruses where pathogen is involved. This is not surprising as in a previous study, the use of molecular diagnostics was particularly important in identifying a pathogen in 90% of culture-negative cases (Khater and Elabd, 2016). Taken together, our findings with these demonstrate the big challenge associated with the identification causative agents in CNS infections, in settings with limited diagnostic capacity like Rwanda. It also supports the hypothesis that, the use of molecular methods could improve pathogen identification for CNS infections. These emerges from this study that, the most common microbial pathogen causing meningitis in all the four referral care hospitals is *C. neoformans,* which account for 71.6% in all cases.

These findings are in line with previous studies in the region and often correlate with the high causative agents in CNS infections in settings with limited diagnostic capacity like Rwanda. It also supports the hypothesis that the use of molecular methods could improve pathogen identification for CNS infections (McCarthy et al., 2006). However, in our study this observation may be due to the fact that the diagnosis of this fungus is very simple and readily available in all hospitals included in our study.

On the other hand, *S. pneumonia* represents the second most common pathogens (9.6%) and was reported as the most common bacteria (33%) isolated in their studies (Campagne et al., 1999). It is evident that pneumococcal meningitis is currently the leading cause of bacterial meningitis in Rwanda as opposed to few cases (1/59) of meningococcal and *H. influenza* type b (1/59) meningitis, which indicate the increased use of both polysaccharide meningococcal and *H. influenza* type b vaccines in routine in Rwanda. Although isolated in small number (20/59), our study revealed no difference in the trend of *S. pneumonia* over 4-years, making difficult the assessment of the recent introduction of the pneumococcal vaccine in childhood immunization program in Rwanda. Our findings on the causal agent of CNS infection differ from that of Minjolle et al. (2002) in Iran and by others, who found *H. influenza* type B to be the most common pathogen in their studies (Mommeja-Marin et al., 2003). The reason for their findings is not clear but the finding of Shaban and Siam (2009) clearly stated that the vaccine had not been introduced at the time of their study, which explain the high isolation rate of *H. influenza* type B (Minjolle et al., 2002).

Although this is a large retrospective review over 4-years, we recognize that its retrospective nature could not allow the use of more pathogen targeted testing for identification of other rare causative agents of CNS infections. This may in part explain the high percentage (91.4%) of negative culture. Nevertheless, the study provides useful baseline data highlighting the major gap in the diagnostic capacity for CNS infection in Rwanda.

Conclusion

This cross sectional descriptive retrospective study shows low diagnostic yield of conventional microbiological methods in the diagnosis of CNS infection as 91.4% which remain undiagnosed. It provides valuable baseline data, which suggest the design of a large prospective study using both conventional microbiological and molecular methods to shed more light on the etiological agent and clinical outcome in patient with CNS infection, to help in the elaboration of better diagnostic strategies for CNS infections in Rwanda. This is a major drawback to effective algorithm for diagnosis, treatment and monitoring of drug resistance, which requires reliable diagnostic testing, highly trained staff and molecular laboratory equipment with adequate maintenance and calibration.

Novel algorithm in conventional and molecular methods could be used to detect the most common aetiologic agents of CNS like DNA/RNA virus, parasites, bacteria and fungi. The molecular method seems attractive in order to improve the detection of causative agents of meningoencephalitis for better evidence use in the treatment of patients in Rwanda.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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