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Etiology and risk factors of meningitis in patients admitted at a Central Hospital in Harare

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Abstract

Objective: To determine etiology and risk factors of meningitis in patients admitted a tertiary referral Hospital in Harare.

Design: Cross-sectional study.

Setting: Tertiary, urban-based referral hospital.

Subjects: Patients suspected of having meningitis admitted at Parirenyatwa hospital were consecutively recruited into the study until sample size accrual.

Main Outcome Measures: (a) Prevalence of pathogens associated with meningitis. (b) Risk factors of meningitis.

Results: Two hundred and ninety six (296) patients with clinically suspected meningitis were recruited into the study, 51.7 % (n=115) were male. Meningitis was confirmed in 20.6% (n=61) cases with the following pathogens, *C. neoformans* 45.9 % (n=28); *S. pneumoniae* 27.9 % (n=17); tuberculosis 4.9 % (n=3); probable viral meningitis 6.6% (n=4) and other bacteria 14.8% (n=9). Patients from crowded households were also more likely to suffer from meningitis than those from sparsely populated households (p<0.001).

Conclusion: *C. neoformans* was the single commonest cause of microbiology positive meningitis. The use of latex agglutination increases the proportion of detected pathogens both fungal and bacterial when used in conjunction with cerebrospinal fluid (CSF) gram stain and culture. *Cryptococcus neoformans* and *S. pneumoniae* are the leading causes of meningitis in patients admitted at Parirenyatwa Hospital.

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Introduction

Meningitis is an infectious disease characterized by inflammation of the membrane surrounding the brain and spinal cord most often caused by infection. There is a wide array of causes of meningitis which include bacteria, viruses, fungi and parasites.¹ Isolation and identification of these etiological agents depends on the availability of ideal medical laboratory facilities and trained personnel. The disease is a serious source of Public Health concern associated with high morbidity and mortality worldwide. An approximated 890 000 cases of meningitis are reported to occur annually worldwide with at least 500 000 of these cases occurring in Africa due to poor socioeconomic conditions. The Pacific countries account for an estimated 210 000 cases with 100 000 in Europe and about 80 000 in the United States of America.^{2,4} Out of the approximated 130 000 annual deaths worldwide, two thirds occur in low income countries like those in Africa affecting mainly children under the age of 15

years.³ These statistics show the disproportionately large burden of meningitis on Africa with more than 50% of cases occurring within this region.

Before the advent of antibiotics, fatality in developing countries was as high as 100%. Published literature currently shows case fatality rates of up to 11% in high income countries such as the USA. This case fatality rate although generally regarded as still too high, represents a considerable decline from previously reported figures of up to 33%.^{5,6} Relatively high case fatality rates of up to 25% have been reported in studies conducted in India, this despite WHO efforts to provide conjugate vaccines to reduce the burden of bacterial meningitis in this part of the world.⁷ Low income countries especially in the African region continue to suffer from unacceptably high case fatality rates and other neurological complications of proportions between 15 and 70% and 10 and 35% respectively.² In Egypt, cases fatality rates of between 8.5 and 55% have been reported with similar figure in South Africa and Zimbabwe of 40% in hospital

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patients.^{8,9}

The Harare City Council reported meningitis amongst five leading causes of death in Harare, the capital city of Zimbabwe accounting for a total of 862 deaths for the year 2008 alone.¹⁰ Reports from Zimbabwe show that the magnitude of the health burden due to meningitis is as high as that carried by other parts of the developing world. However, there is limited published data on the etiology of meningitis. The routine clinical laboratory work in the Public Health Laboratories in Zimbabwe has generated data over the years on pathogenic agents of meningitis but this may not represent a true prevalence due to various diagnostic challenges. The country has experienced economic decline for more than a decade. The laboratory diagnostic services in central hospitals have been affected through loss of experienced personnel and general lack of laboratory utilities to support accurate diagnosis of meningitis. The importance of knowledge on meningitis etiology for both patient management and policy formulation raises the need for well controlled studies to determine the prevalence of pathogens in the country.

Materials and Methods

Culture and Microscopy.

Patients who were identified by the attending doctor on admission to Parirenyatwa Central Hospital as clinically suspected of having meningitis were consented and consecutively enrolled into the study between June and August 2012. Demographic and clinical data was collected through participant interviews and clinical notes review. Cerebrospinal Fluid (CSF) samples were aseptically collected by lumbar puncture and delivered to the laboratory at ambient temperature for processing within an hour of collection. A cell counting was done on a portion of whole CSF sample. The samples were then centrifuged at 3,000 rpm for 10 minutes and the supernatant stored at 2-8 °C pending serological test. The CSF sediment was inoculated on Sheep Blood agar (BA, OXOID CM0055), Chocolate Agar (CA, OXOID CM0055,) and Sabouraud Agar (SBA) and incubated at 37°C in 5% CO₂. Culture plates were examined daily for up to 72 hrs and isolated organisms were identified using a combination of gram stain reaction and standard biochemical tests. The sediment was also used to prepare gram smear slides for presumptive identification of bacteria and Ziehl-Neelsen stain for *Mycobacterium tuberculosis* as well Leishman staining for white cell differential analysis under a light microscope.

Latex Agglutination Testing.

The CSF supernatant was tested for the presence of polysaccharide antigens for *H. influenzae*, *N. meningitidis*, *S. pneumoniae*, Group B *Streptococci* and *E coli* using a Latex Agglutination Test (LAT) (Wellcogen[®] Bacterial Antigen kit R30859602 ZL26)

and *Cryptococcus neoformans* polysaccharide antigen using Cryptococcal Antigen Latex Agglutination System (CALAS[®]).

Antibiotic Susceptibility Testing.

Antimicrobial susceptibility testing was done on pure culture isolates using the disk diffusion method using Mueller Hinton agar (OXOID CM0337) following Clinical and Laboratory Standards Institute (CLSI) guidelines.¹¹ The following antibiotic discs (Mast) were used: Erythromycin 15ug, Gentamycin 10ug, Tetracycline 30ug, Ceftriaxone 30ug, Cefuroxime 30ug, Ciprofloxacin 5ug, Ampicillin 10ug, Oxacillin 1ug, Clindamycin 2ug, Chloramphenicol 30ug and Ceftazidime 30ug. The accuracy of the disc diffusion testing was monitored through the use of control strain *Staphylococcus aureus* ATCC 29213. The acceptable limits for inhibition zone diameters for quality control strain were derived from CLSI guidelines.¹¹

Case Definition.

Cryptococcal meningitis was defined as when capsulate yeast cells are seen under the microscope after India ink staining, positive culture for *Cryptococcus neoformans* or positive Cryptococcal antigen test reaction. Bacterial meningitis was reported when bacteria was detected on gram stain, culture or positive latex agglutination with Wellcogen[®] antigen test kit on CSF specimen. Tuberculous meningitis was defined as when acid-alcohol fast bacilli were seen under the light microscope on ZN stained CSF sediment. Probable viral meningitis was reported when there were no bacteria or fungi in CSF after all staining procedures and microscopic examination of CSF samples with cell counts 10cells/mm³ or greater with lymphocytes being the predominant cell type on Leishman staining.

Ethics

The permission to carry out the study was obtained from the Hospital Clinical Director's Office and Parirenyatwa Joint Research Ethics Committee (JREC).

Informed consent was obtained from participants by the researcher following a process where study objectives were clearly explained to the patients. Patients who could not comprehend English language had the informed consent process administered in Shona. Where patients were below the legal age of consent (16 years), assent was sort from the parents or legal guardian. All records generated in the study were kept confidential by the researcher and no names were included at the data entry stage, laboratory generated accession numbers were used as identifiers.

Statistical Analysis.

Data was entered into Microsoft excel 2007 spreadsheet and imported into STATA version 11 Data editor. Continuous data was checked for normality and where it was found to be normal, mean was used as summary statistic, median lower and upper quartile

ranges were reported when STATA analysis showed that data was not normal. Data was tabulated to give frequencies of demographic characteristics, risk factors as well as pathogenic organisms. Univariate and Multivariate logistic regression analysis was performed in STATA version 11 for determination of risk factor association with meningitis. A p value of 0.25 was used as a cut off for selection of variables from univariate analysis for inclusion in the multivariate model. Odds ratios, p value and 95% confidence intervals were tabulated and used to interpret results where $p < 0.05$ was considered to be statistically significant.

Results

Two hundred and ninety six (296) study participants clinically suspected of having meningitis were recruited into the study between June and August 2012. There were no eligible participants who declined to participate in the study. Out of the total enrolled 296 participants, 153 (51.7%) were male.

The median age of the total study population was 31.5yrs and the lower and upper quartiles were 8.0 and 42.0. Eighty Three (28.6%) of the participants were below the age of 18 years with 24.5% being below 5 years of age. 41.7% (n=121) of study participants were above 36 years of age. Table I summarizes the demographic and clinical characteristics of 290 recruited participants.

Table I: Summary of demographic and clinical characteristics of study participants.

Characteristics	No. of patients (%)
Age distribution of patients (yrs)	n=290*
0-5	71 (24.5)
6-11	6 (2.1)
12-17	6 (2.1)
18-23	15 (5.2)
24-29	34 (11.7)
30-35	37 (12.8)
36+	121 (41.7)
Gender	n=296
Male	153 (51.7)
Female	143 (44.3)
Symptoms of patients at admission	n=294*
Headache	124 (42.2)
Neck stiffness	139 (47.28)
Fever	173 (58.64)
Fits	46 (15.6)
Confusion	72 (24.4)
Blindness/blurred vision	11 (3.7)
Paralysis	8 (2.7)
Photophobia	36 (12.3)
Vomiting	101 (34.5)
Cough	90 (30.6)
Sought health services before coming to hospital	211 (71.8)
Antibiotics administered before admission	180 (61.0)
HIV infected	93 (31.6)
HIV uninfected	29 (9.8)
HIV status unknown	173 (58.45)
Diabetes mellitus	10 (3.4)

*Ages could not be determined for 6 participants and symptoms at admission for 2 patients were not available from the patient's notes.

The majority of participants were over the age of 36

years with almost equal female representation. More than half (58.6%) of all participants presented with fever at admission.

Table II: Distribution of risk factors in study participants.

Risk factor	Risk factor distribution amongst study participants		
	Meningitis (%) n=61	No meningitis (%) n=235	p value
Gender:			
Male	34 (55.7)	119 (50.6)	0.566
Female	27 (44.3)	114 (48.5)	
Marital status:			
Married	21 (34.4)	96 (40.9)	0.038
Unmarried	30 (49.2)	71 (30.2)	
Widowed	1 (1.6)	3 (1.3)	
Employment status:			
Employed	15 (24.6)	44 (18.7)	0.033
Unemployed	31 (50.8)	128 (54.5)	
Income status:			
<US\$100/month	31 (50.8)	138 (58.7)	0.031
>US\$100/month	14 (22.9)	34 (14.5)	
Accommodation:			
<5 people/household	12 (19.7)	187 (79.6)	<0.001
>5 people/household	39 (63.9)	34 (17.4)	
Smoke:			
Yes	23 (37.7)	18 (7.7)	<0.001
No	28 (45.9)	172 (73.2)	
Alcohol:			
Yes	30 (49.2)	23 (37.7)	<0.001
No	27 (44.3)	167 (71.1)	
HIV status:			
Positive	29 (47.5)	64 (27.2)	0.006
Negative	7 (11.5)	22 (9.4)	
Status unknown	25 (40.9)	147 (62.6)	

*Percentages were calculated on the denominator of cases with and without meningitis.

There was a statistically significant difference in the distribution of all risk factors except gender between participants with and without meningitis based on clinical and laboratory diagnosis.

Comparative analysis of LAT and Culture

The routine combination of gram reaction and CSF culture method isolated 29.5% (n=18) of all cases of meningitis. Latex agglutination testing identified 59.0% (n=36). Table III shows tabulated results of detected pathogens by laboratory method.

Table III: A comparative analysis of sensitivity of routine CSF culture methods and latex agglutination techniques.

Organisms isolated/ Detected from CSF	Culture n (%)	Latex Agglutination n (%)	No. detected by LAT alone n(%)
<i>S. pneumonia</i>	4 (6.6)	17 (27.9)	13 (21.3)
<i>E. Coli</i>	0	0	0
Group B streptococci Coagulase negative	1 (1.6)	2 (3.2)	1 (1.6)
<i>Staphylococcus</i>	2 (3.2)	0	0
<i>L. monocytogenes</i>	1 (1.6)	0	0
<i>K. pneumoniae</i>	1 (1.6)	0	0
<i>H. influenzae</i>	0	0	0
<i>S. maltophilia</i>	1 (1.6)	0	0
<i>N. meningitidis</i>	0	2 (3.2)	2 (3.2)
<i>C. neoformans</i>	8 (13.1)	28 (45.9)	20 (32.8)
Total	18 (29.5)	49 (80.3)	36 (59.0)

The addition of Latex agglutination test resulted in 36 more pathogens being identified that had not been detected by either culture or microscopy techniques. There were 3 cases of Tuberculous Meningitis detected in this study representing 4.9% of all meningitis cases reported.

Antibiotic Susceptibility: All isolates of *S. maltophilia* (1), *L. monocytogenes* (1) and coagulase negative *Staphylococcus* (2) grown were susceptible to the antibiotics tested. 100% (4) of *S. pneumonia* isolates were susceptible to Ceftriaxone, Cefuroxime, Ciprofloxacin and Ampicillin while 25% (1) showed resistance to Erythromycin and Tetracycline.

Multivariate Logistic Regression Analysis: Multivariate regression analysis was performed for HIV status, socioeconomic status, age group, accommodation and marital status to test for association with meningitis. $p < 0.05$ was considered to represent a statistically significant observation. Table IV shows results of multivariate regression analysis.

Table IV: Multivariate regression analysis results for meningitis and associated risk factors.

Risk factor	Confirmed meningitis				p value
	N	%	OR	95%	
Age (Years)					
0-5	8	13.1	1	*	*
6-17	3	4.9	2.29	0.51-10.1	0.28
>18	50	82	2.44	1.05-5.45	0.03
Marital status					
Married	21	34.4	1	*	*
Unmarried	30	49.2	2.82	1.24-6.40	0.010
Widowed	1	16.4	2.22	0.21-23.8	0.510
Income (monthly USD)					
<100	34	55.7	1	*	*
>100	18	29.5	1.57	1.05-2.37	0.030
Accommodation					
<2	2	3.3	1	*	*
2 or more	59	96.7	4.98	2.67-7.50	<0.001
Marital status					
Yes	21	24.5	1	*	*
No	9	14.5	1.53	0.92-2.55	0.101
HIV status					
Yes	7	6	1	*	*
No	29	19.3	1.42	0.55-3.71	0.470

Living in crowded accommodation of 2 or more in a household was significantly associated with meningitis.

Discussion

The study recruited 296 participants and 51.7% (n=153) were male. The slight difference in proportions of males and females was not statistically significant ($p = 0.24$). The results of the current study show a low ratio of clinically suspected meningitis to laboratory confirmed meningitis. Confirmed HIV infection in this study was 31.6% with status undetermined in 58.5% of participants. The relatively high HIV prevalence and manifestation of this pandemic may also be contributing to an array of symptoms that might complicate clinical diagnosis of meningitis.^{8,12}

The study detected pathogens in 61 (21%) cases out of the total enrolled number of 296 participants. This figure is slightly lower than results reported from a 1994 prospective study done at Parirenyatwa and Harare Central Hospitals which confirmed meningitis in 49.3% of suspected cases.⁸ It is however important to note that the 1994 study constituted predominantly adult participants. A study done in a high HIV incidence setting in South Africa recorded a lower confirmation rate of 16.5%.¹² This data although obtained at different time points could point out to variable detection rates arising from different laboratory methodologies. The South African study did not use Latex agglutination techniques in addition to standard diagnostic methods as in the current study.

Data collected through participant interviews and review of hospital records show that 71.8% of participants had been to other health institutions prior to admission and of these 61% had taken antibiotics prior to hospitalization at Parirenyatwa Group of Hospitals. This observation probably explains the low pathogen detection rate by CSF culture and gram staining technique which was 26.3% in this study. Sensitivities of both culture and gram stain have been reported to decrease to between 40 and 60% in patients on antibiotics prior to lumbar puncture.¹²⁻¹³ A 2006 Bangladesh study reported 75.5% of patients being exposed to antibiotics prior to hospital admission and went on to record 40% pathogen detection by LAT as opposed to 13.3% by gram stain and CSF culture.¹³ These results clearly show the negative impact of antibiotics on the diagnosis of meningitis based on pathogen detection from CSF specimens. Results show that *C. neoformans* accounted for 45.9% of all confirmed cases of meningitis over the 3 month duration of this study confirming it as the leading cause of meningitis in Zimbabwe. A study carried out in Zimbabwe by Hakim *et al* in 1994 reported *C. neoformans* as the causative agent in 45% of all confirmed meningitis cases.⁸ The figure from the Hakim study was reproduced in the current study, an indication that the prevalence of fungal meningitis has

not changed significantly over the last decade.

A 2008 study in South Africa reported *C. neoformans* as causing 63% of all meningitis cases and Tuberculous Meningitis (TBM) was second most frequently diagnosed accounting for 28% of patients.¹² The results of the present study although showing lower proportions of both cryptococcal Meningitis (CM) and TBM, they are in keeping with data from studies done in Central and Southern Africa over the past decade. A frequency in the range 27-45% has generally been reported in this part of the world.^{8,12,14}

The proportion of TBM in the current study was much lower than what has been reported in most South African studies but do not differ much from results shown by studies done from the rest of Africa which reported TBM frequency in the range 1-17%.^{8,14} The figures coming from the current study seem to be an underestimate of the true burden of TBM and this could be due to under diagnosis by routine laboratory investigations that were used in the study. The sensitivity of ZN in determining TBM is very low and application of more sensitive procedures such as polymerase chain reaction (PCR) techniques could have yielded higher pathogen prevalence.

S. pneumoniae was the second most common pathogen detected in the study representing 27.9% (n=17). The comparison between results of this and the study by Hakim *et al* which utilized similar laboratory methods suggests an increase in pyogenic meningitis when considering percentage proportions. Hakim *et al* reported *S. pneumoniae* as constituting 15/200 (7.5%) of confirmed meningitis. A review of data considering absolute numbers alone show striking similarities in the absolute numbers of the two studies with the current study reporting 17 cases of *S. pneumoniae*.⁸ It is however imperative to note that the 1994 Zimbabwean study was conducted on an adult population at the two biggest referral hospitals in the country over a period of 10 months whereas the current study included all age groups and was done over 3 months. The prevalence of cases of *S. pneumoniae* may have gone up given that more cases were detected over a shorter period of time at only one of the sites involved in the previous study. However, there may be seasonality to the incidence of *S. pneumoniae* causing an over-estimate when three months are projected to twelve months.

A 1997 Mozambican study reported *S. pneumoniae* as accounting for 21% of meningitis cases; *H. influenzae* was 33%.¹⁵ The present study did not detect any cases of *H. influenzae* which is rather surprising given that use of vaccines remains minimal in Zimbabwe.

The application of latex agglutination technique in this study significantly increased the frequency of bacterial pathogens detected which is in line with published literature from studies done elsewhere. The sensitivity of latex agglutination in culture negative CSF samples was reported to be as high as 54.5% in studies elsewhere.¹⁶ The present study reports additional 59.0% bacterial and fungal pathogens detected by LAT.

Notably though, out of the five possible detectable strains of bacteria, only three were detected in the study, an observation that is worth consideration in any future decisions to adopt use of LAT in a similar setting.

The main limitation of LAT for detection of bacterial pathogens is the fact that it is only positive in the presence of specific polysaccharide surface antigens for *H. influenzae*, *N. meningitidis* A, C, Y, W-135, *Streptococcus pneumoniae*, Group B *Streptococci* and *E. Coli* while other bacterial species remain undetected for example, *Klebsiella* and *Staphylococcus* species are not detectable by this kit. The application of LAT however remains useful in a setting of high antibiotic usage like Zimbabwe. The use of broad spectrum antibiotics is recommended for the treatment of bacterial meningitis and the timely identification of specific bacterial pathogen to guide specific antibiotic therapy would be beneficial to minimize emergence of resistant strains.

This study showed that age and poor socioeconomic status are statistically significantly associated with meningitis. A study done in Egypt showed that smokers were 3 times more likely to develop meningitis as compared to non smokers, the same study also reported that neonates below the age of 1 year were 8 times more prone to meningitis when compared to infants above 1 year of age.² The predisposing role of HIV to meningitis is well documented in other studies done in Africa.^{8,12} The current study failed to reproduce this finding and this could be attributable to the failure to determine HIV status in a significant proportion of the study participants (58.5%). The study however demonstrated the known association between cryptococcal meningitis and HIV (p=0.001) with results showing that 78.6% of all cases were confirmed HIV positive patients.

The failure by this study to establish known relationships between meningitis and certain risk factors could be due to inaccuracies in data collected resulting from social desirability bias. The participant responses to some of the questions such as smoking and alcohol consumption patterns could have been driven by the desire to provide answers deemed "correct" by the interviewer.^{2,6,13,17} The neurologic complications of meningitis made it difficult in certain instances to get coherent answers from participants and there was over reliance on documented clinical notes and this made it difficult to get all the necessary information. All isolates identified in this study were susceptible to Ceftriaxone which is one of the recommended drugs of choice according to the 5th Essential Drugs List and Standard Treatment Guidelines for Zimbabwe (EDLIZ).¹⁸

Generalisability

The population from which the this study derived its participants is a highly selected group of patients who would have been referred from various primary health care facilities and thus may have certain characteristics that are not consistent with the rest of the population.

The results can however be used to guide policy formulation and guide empiric antibiotic administration to meningitis patients in Zimbabwe since the referred patients come from all parts of the country. A broad based study recruiting patients from all health care facilities across the country and carried out over a longer period offers the best chance to generate data which gives a true representation of the prevalence of pathogens. The fact that data collection for this study was conducted over a three month period also makes it prone to seasonal variations which would again limit generalisability of findings.

Policy Implications

The study results offer objective evidence on the extent to which patients admitted at referral hospitals such as Parirenyatwa would have been exposed to antibiotic medication at primary health care facilities. The high proportions of patients exposed to antibiotics prior to CSF collection reported in this study calls for policy review on the laboratory diagnostic algorithm to co-opt methodologies that can improve diagnosis of partially treated meningitis given the known limitations of CSF culture and Gram stain for this group of patients. The prevalence of bacterial pathogens reported in this study gives an insight into the country's vaccine requirements as preventive strategies.

Further Research

The study's relatively small sample size and short duration raises the need for a more encompassing large study to determine a broader spectrum of pathogens. The true burden of tuberculous and viral meningitis could not be established in this study due to complex structural and financial requirements needed to set up the necessary laboratory techniques to achieve accurate diagnosis. It thus becomes imperative to set up research studies to specifically address the prevalence of tuberculous and viral meningitis especially with the high HIV prevalence in the country. There is also need to conduct further research to assess risk factor association with meningitis and the study design should particularly address ways of extracting sensitive information on social and behavioral characteristics of patients.

Recommendations

Results of this study provide an insight into the usefulness of LAT given that most patients presenting at Parirenyatwa Group of Hospitals would have been exposed to medication at primary health facilities. Observations and results obtained in this study give rise to the following recommendations

- Use of gram staining and culture complemented by LAT would greatly improve both detection and identification of bacterial pathogens.

- This study although managing to show significant association between some risk factors with meningitis raises the need for a bigger cohort study to determine causality.

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