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Microbiology of HIV associated bacteraemia and diarrhoea in adults from Nairobi, Kenya

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SUMMARY

We undertook a retrospective descriptive comparison of the spectrum of pathogens responsible for bacteraemia and diarrhoea in HIV antibody positive and negative patients over 4 years (1988–92), in Nairobi, Kenya. The study population was recruited from primary to tertiary centres of clinical care and consisted of 2858 adults (15 years or older). There were 415 significant blood culture isolates, 192 from 1785 HIV negative patients and 223 from 953 HIV positive patients. There were 233 significant faecal isolates, 22 from 115 HIV negative patients and 211 from 531 HIV positive patients. The most common pathogens detected in blood were *Streptococcus pneumoniae* and *Salmonella typhimurium* and in faeces *Shigella flexneri*, *S. typhimurium* and *Cryptosporidium parvum*. The agents causing illness in HIV positive patients in Nairobi are similar to those prevalent in the HIV negative community and the investigation of a febrile illness with or without diarrhoea in an HIV positive patient should reflect this.

INTRODUCTION

Bacterial infections are the predominant cause of HIV-associated morbidity and mortality [1, 2] in Africa and frequently present as fever or diarrhoea [3]. Studies from Africa have investigated HIV-associated diarrhoeal disease but have been limited either to specific populations, for example, children [4, 5] or to an extensive search for a particular organism or group of organisms [6, 7]. Investigations into the microbiology of systemic sepsis have been limited in much the same way as with diarrhoeal disease, with searches for specific pathogens, for example mycobacteria [8, 9]. The reasons for the lack of data from Africa on the microbiology of HIV-associated illness are two-fold. Firstly, the initial reports of HIV-associated morbidity focused on pathogens that are seen in late stage disease and which are of limited significance in Africa [10]. Secondly, there are few laboratories in areas of high HIV prevalence in Africa that have the capability to diagnose accurately the wide range of infectious diseases present.

The Wellcome Trust–Kenya Medical Research Institute microbiology laboratory has examined blood cultures and stool specimens from a broad spectrum of adult patient groups as part of a series of studies into the infectious causes of morbidity associated with HIV, since 1988. In order to examine as fully as possible the aetiology of HIV-associated morbidity and to compare it with the range of pathogens causing disease in the HIV negative population, we undertook a retrospective analysis of all stool specimens received over a 45-month period and all blood cultures received over a 36-month period, from those patients whose HIV status was known.

METHODS

Patients

Blood and stool specimens were obtained from adult (15 years or older) patients participating in nine

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Table 1. *Component studies used in the retrospective analysis with the number of patients presenting blood and stool specimens*

Study	Year(s)	Patient groups [reference]	Patients presenting		% HIV positive	% Female
			Blood	Stools		
1	1988–9	Acute medical admissions [11]	600	0	17	41
2	1989–91	Tuberculosis in and outpatients [12]	542	166	55	37
3	1989	Acute meningitis [13]	62	0	19	36
4	1989–91	Admitted with abortion*	926	0	10	100
5	1989–92	Sex workers	366	360	90	100
6	1990	Pelvic inflammatory disease	42	0	19	100
7	1990	Acute pneumonia admissions	200	0	45	30†
8	1992	Chronic diarrhoeal disease	0	105	79	54
9	1992	Acute medical admissions	0	15	53	42

* Also contains pregnant controls.
† Estimate based on representative population.

studies which ran from November 1988 to October 1992. The clinical findings and detailed methodology of some of the individual studies have been published (Table 1). Blood cultures were received from seven studies and stool specimens from four studies. Each study was designed to investigate an aspect of possible HIV-associated disease by looking either at a particular presentation such as meningitis or pneumonia, or at a particular group of patients such as those with tuberculosis or sex workers. The blood and stool cultures were analysed by morbidity event, with stools being requested from patients with self-reported diarrhoea and blood being drawn from every patient whatever the clinical presentation. A new event was defined as one occurring at least 1 week after the initial event. One week was selected as it allowed time for any causal pathogen to be isolated and for a response to treatment to have occurred. Informed consent was obtained for venepuncture and HIV testing prior to recruitment and each of the studies was approved by the Kenya National Ethical Committee.

Microbiology

All samples were cultured on the day of collection using Oxoid (Unipath, Basingstoke, UK) media throughout. Media were incubated in air at 37 °C for 18 h unless otherwise stated.

Culture for *Salmonella* spp. and *Shigella* spp. was carried out by direct inoculation onto xylose lysine desoxycholate medium (XLD), brilliant green agar (BG), and selenite F broth. After incubation the broth was subcultured onto both XLD and BG agars. Campylobacter blood-free medium, with cefoperazone selective supplement, was directly inoculated and incubated micro-aerophilically at 37 °C for 48 h before examination. Cefsulodin-irgasan-novobiocin (CIN) medium was used to culture *Yersinia* spp. after direct inoculation and after prior cold enrichment in phosphate buffered saline at 4 °C for 14 days. CIN plates were incubated at 30 °C for 24 h before examination. Direct inoculation onto TCBS medium and enrichment in alkaline peptone water were used to culture vibrios. Detection of *Clostridium difficile* was either by culture using *C. difficile* selective agar, supplemented with D-cycloserine and cefoxitin, incubated anaerobically at 37 °C for 48 h or by direct toxin detection in MRC 5 human fibroblast cell lines. Investigation for *Yersinia* spp., *Vibrio* spp. and *C. difficile* was only undertaken when workload and resources permitted.

Cryptosporidium parvum was sought using phenol-auramine stained smears examined by fluorescent microscopy. Slides giving equivocal results were re-examined after staining with the modified Ziehl–Neelsen method. Samples were examined for the presence of ova, cysts and parasites either by direct examination of a wet preparation in saline and iodine or following formalin-ether concentration of the sample.

Blood cultures were performed by inoculating *c.* 5 ml of venous blood into 50 ml brain heart infusion broth enriched with 5% CO_2. After incubation at 37 °C, any turbid broth cultures were subcultured onto both horse blood and chocolate agar plates incubated in air, anaerobically and in 5% CO_2 for up

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to 5 days. Broth cultures which remained clear for 7 days were subcultured terminally.

Identification of isolates was by standard bacteriological techniques and confirmation of identification, along with phage typing and serotyping where appropriate, were carried out by the Public Health Laboratory Service, UK.

Serology

HIV testing was performed using a range of HIV enzyme immuno-assays (EIA): Wellcozyme Recombinant, (Wellcome Diagnostics, Dartford, UK), Dupont (Dupont de Nemours, Delaware, USA), Wellcozyme HIV 1+2 (Wellcome Diagnostics, Dartford, UK), Enznergost HIV 1+2, (Behringwerk AG, Marburg, Germany). Only one test was performed routinely on each serum sample. Repeat serum samples were requested whenever subsequent blood cultures were taken, and these were tested as above. Any equivocal sera were retested using a different EIA to the original.

RESULTS

Blood cultures

A total of 4629 blood cultures was received from 2738 patients within the seven studies. There were 1899 morbid events with 2006 blood cultures from 1785 HIV negative patients and 1785 morbid events with 2623 blood cultures from 953 HIV positive patients.

There were 415 significant blood culture isolates, 192 from HIV negative patients and 223 from HIV positive patients; 20 blood cultures had more than one significant organism. A summary of significant blood pathogens is presented in Table 2.

The predominant pathogens from blood were *Streptococcus pneumoniae* and *Salmonella typhimurium*. Amongst the Gram-negative bacteria *S. typhimurium* was the single most common isolate, with phage types 56 (30%) and 193 (16%) being the most common. *Escherichia coli* was the second most common isolate and was most frequently associated with urinary tract infections. The most common *Salmonella typhi* phage types were E1 (50%) and A (36%).

Both *Brucella melitensis* were biotype 1 and one patient also had *B. melitensis* isolated from a breast abscess. Both *Haemophilus influenzae* were non-capsulated. All *Neisseria meningitidis* were isolated during an 18-week period in mid 1989 and all were group A.

Streptococcus pneumoniae was a common isolate from blood amongst HIV negative as well as HIV positive patients and was most frequently associated with pneumonia; six patients had more than one pneumococcal bacteraemic event and all were HIV positive. *Staphylococcus aureus* bacteraemia was com-

Table 2. *Blood culture isolates by HIV status. The total number of isolates exceeds the number of significant events because some blood cultures contained more than one isolate*

Isolates	Number of positive events	
	HIV Negative	HIV Positive
Salm. typhimurium	5	56
Salm. typhi	8	6
Other *Salmonella* spp.	2	4
Escherichia coli	7	15
Shigella flexneri	0	2
Enterobacter spp.	8	0
Morganella morganii	1	2
Citrobacter diversus	1	0
Klebsiella pneumoniae	0	1
Serratia liquefaciens	1	0
Pasteurella spp.	0	3
Acinetobacter spp.	1	5
Pseudomonas spp.	2	3
Gardnerella vaginalis	4	0
Brucella melitensis	0	2
Haemophilus influenzae	1	1
Neisseria meningitidis	12	1
Aeromonas spp.	2	1
Campylobacter coli	0	1
Vibrio fluvialis	1	0
Strep. pneumoniae	25	58
Beta-haem. streptococci		
Group A	4	2
Group B	13	2
Group C	2	0
Strep. milleri group	2	1
Viridans streptococci	9	2
Enterococcus spp.	1	2
Aerococcus viridans	6	5
Staph. aureus	16	14
Clostridium spp.	8	11
Bacteroides spp.	23	14
Anaerobic streptococci	25	2
Veillonella spp.	1	0
Fusobacterium spp.	1	0
Cryptococcus neoformans	0	7

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Table 3. *Stool isolates by HIV status. Some stools had more than one bacterial pathogen isolated*

Isolates	Number of positive events	
	HIV Negative	HIV Positive
Bacteria		
*Salmonella typhimurium**	3	40
Salmonella havana	0	2
Salmonella enteritidis	1	1
Salmonella typhi	0	2
Salmonella braenderup	1	1
Other *Salmonella* spp. (1 each)	1	12
*Shigella flexneri**	9	49
Shigella sonnei	3	15
Shigella boydii	1	15
Shigella dysenteriae	1	5
Campylobacter coli†	0	3
Campylobacter jejuni	0	4
Ova, cysts, parasites		
Strongyloides sp.‡	0	3
Ascaris lumbricoides	6	21
Schistosoma mansoni	2	6
Tricuris trichiura	2	12
Hookworm	12	61
Taenia spp.	0	3
Entamoeba histolytica	0	14
Giardia lamblia	2	3
Cryptosporidium parvum§	0	45

* 1173 (142 from HIV negative patients) were cultured for *Salmonella* and *Shigella* spp.
† 1171 (140 from HIV negative patients) were cultured for *Campylobacter* spp.
‡ 1128 (139 from HIV negative patients) were examined for ova, cysts and parasites.
§ 1167 (142 from HIV negative patients) were examined for *Cryptosporidium parvum*.

monly associated with focal sepsis, predominantly soft tissue.

Fifteen of the 19 *Clostridium* spp. were *C. perfringens* and the majority of the anaerobes were isolated from women presenting with abortion.

Stool cultures

A total of 1301 stool samples were received for examination from 646 patients within four studies. There were 175 samples from 142 events from 115 HIV negative patients and 1126 samples from 935 events from 531 HIV positive patients. There were 233 significant faecal isolates, 22 from HIV negative patients and 211 from HIV positive patients. Only 21 (1·9%) had more than one recognized cause of diarrhoea present, most commonly *Shigella* spp. in association with *Cryptosporidium parvum*. One HIV seropositive patient grew both *Shigella flexneri* and *Shigella boydii* from the same sample. The presence of helminth ova or larvae was not regarded as being the cause of diarrhoea, but as incidental. A summary of significant stool pathogens is presented in Table 3.

The most common identifiable bacterial causes of diarrhoea among our patients were *Shigella* spp. and *Salmonella* spp. *S. typhimurium* accounted for 67% of all salmonella infections, with phage types 56 (28%) and 193 (19%) being the most common. *Shigella flexneri* caused 59% of all shigella infections with the commonest serotypes being 1b (36%) and 2a (26%).

Clostridium difficile, *Yersinia enterocolitica* or *Vibrio* spp. were not detected during the examination of stools from 254 patients (152 for *C. difficile*) early on in the study.

Ova, cysts and parasites, other than *Cryptosporidium parvum*, were seen in over 12% of stool samples but *Giardia lamblia* and *Entamoeba histolytica*, regarded as the only amoebic causes of diarrhoea in our study, were seen in only 1·7% of samples. No attempt was made to discriminate between *Cryptosporidium parvum* and *Cyclospora cayetanensis*.

DISCUSSION

Laboratory services can play a vital part in individual patient management in developing countries and be of long-term benefit in generating epidemiological data which may help to shape public health strategies. This retrospective analysis of blood and stool pathogens from patients with a high frequency of HIV infection is one of the largest reported from Africa. It demonstrates the wide range of organisms causing illness amongst a poor urban African community but does not attempt to assign to any specific pathogen a greater or lesser association with HIV infection. This has been clearly demonstrated in other studies [11, 14–16]. The incorporation of clinical data has been avoided because of the disparate nature of the patient groups within this analysis. Each patient group had different admission criteria, with medical contact and intervention occurring at different points. In some groups of patients several specimens were obtained from each patient whereas in other groups sampling was limited to one specimen of a particular type. The groups were also very different in sex and

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age as well as being recruited over a 4-year period in which our management of clinical illness changed considerably. The intention of this analysis was to describe the spectrum of agents causing illness amongst the HIV positive population and to compare it with the spectrum of agents prevalent amongst the HIV negative population.

Despite the large number of patients included in the analysis, few stool isolates were found within the HIV negative population compared with those isolated from the HIV positive group. Diarrhoea was relatively uncommon and short-lived amongst HIV negative patients. The most common bacterial faecal pathogens seen in a Nairobi hospital study reflected our findings, although these were not analysed by patient HIV status [17].

Only two infectious agents, *Cryptosporidium parvum* and *Cryptococcus neoformans*, were found in substantial numbers of HIV positive patients but not at all amongst HIV negative patients. We found isolates of cryptosporidium to be unevenly distributed over the study period with 9 cases seen in one 8-week period, yet the preceding 9 cases were seen over 31 weeks and the following 9 cases over 57 weeks. This cluster occurred following a long dry period in Nairobi and was probably associated with disturbances to the municipal water supply; outbreaks associated with drinking water have already been well documented in the USA and UK [18, 19]. All *Cryptococcus neoformans* were variety *neoformans* as are nearly all isolates from HIV positive patients [20].

Resource-poor countries need to provide relevant testing for locally common pathogens and avoid any unnecessary investigations. For example, early in the studies it became clear that routine screening for *Vibrio* spp., *Yersinia* spp. and *Clostridium difficile* was not necessary on all stool samples and could be discontinued. It is therefore important to define the nature of the agents causing disease in the community. Investigation for opportunistic pathogens associated with late stage HIV disease may benefit small numbers of HIV infected individuals but should not be considered essential. Routine investigations and microbiological examination are sufficient to diagnose disease in the vast majority of HIV positive patients. In Nairobi, with two exceptions, the spectrum of agents associated with disease in HIV positive patients is identical to that of HIV negative patients. The investigation and immediate management of a febrile illness with or without diarrhoea in an HIV positive patient should reflect this.

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