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Bacteria isolated from bloodstream infections at a tertiary hospital in Dar es Salaam, Tanzania – antimicrobial resistance of isolates

S Moyo, S Aboud, M Kasubi, S Y Maselle

Objective. A bloodstream infection (BSI) is a life-threatening condition. We studied the causative agents of BSIs and antimicrobial susceptibility patterns of bacterial isolates at Muhimbili National Hospital (MNH) in Dar es Salaam, Tanzania.

Methods. A retrospective analysis of blood culture results obtained at MNH from January 2005 to December 2009 was done. Blood culture isolates judged to be clinically significant and antimicrobial susceptibility results of the bacteria were included. The frequencies and proportions of bacteria isolated and antimicrobial susceptibility results were analysed and compared using Pearson's chi-square test and Fisher's exact test where applicable, or the Mann-Whitney U-test.

Results. A total of 13 833 blood cultures were performed. Bacterial pathogens were detected in 1 855 (13.4%), Gram-positive bacteria (1 523; 82.1%) being significantly more prevalent than Gram-negative bacteria (332; 17.9%) (*p*=0.008). The most common bacterial pathogens isolated were coagulase-negative staphylococci (CoNS) (1 250; 67.4%), *S. aureus* (245; 13.2%), *Escherichia coli* (131; 7%) and *Klebsiella* spp. (130; 7.0%). All bacteria isolated showed high resistance to penicillin G (70.6%), tetracycline (63.8%), cefotaxime (62.5%) and ampicillin (62.3%). Moderate to high resistance was seen against chloramphenicol (45.2%), erythromycin (35.0%), ciprofloxacin (29.3%), co-trimoxazole (25.0%) and gentamicin (23.5%). Of *S. aureus* isolates, 23.3% were resistant to methicillin.

Conclusions. CoNS accounted for two-thirds of the bacterial pathogens isolated. High-level resistance was seen to first-line and inexpensive antimicrobial agents. Routine screening for extended-spectrum beta-lactamase production and methicillin resistance among Gram-negative rods and *S. aureus* from blood cultures should be instituted to monitor spread of multidrug-resistant isolates.

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A bloodstream infection (BSI) is a life-threatening condition that may be complicated by septic shock and death. BSI due to bacteria is an important cause of morbidity and mortality.[1] Gram-negative bacteria have been associated with more deaths than Gram-positive bacteria.[1] Mortality due to septic shock can be as high as 60% despite treatment. A better understanding of the spectrum of pathogens causing BSI is crucial for prompt management of patients, as antimicrobial therapy greatly influences the outcome of patients with BSI.[1]

Over the past 20 - 30 years there have been significant changes in the causation and epidemiology of BSI. While *Staphylococcus aureus* and *Escherichia coli* continue to be the most common causative agents, there has been a substantial increase in BSI due to coagulase-negative staphylococci (CoNS).

Recent studies have reported an increasing frequency of antimicrobial resistance among microbial pathogens causing BSI in Tanzania and elsewhere,[2,3] and several classes of antimicrobial agents have become less effective as a result of the emergence of antimicrobial resistance, often caused by the selective pressure of antimicrobial usage. The important emerging problems are resistance to extended-spectrum cephalosporins and fluoroquinolones in the

Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

S Moyo, MD, MMed (Microbiology and Immunology)

S Aboud, MD, MMed (Microbiology and Immunology)

S Y Maselle, MD, PhD

Department of Laboratory Services, Microbiology Section, Central Pathology Laboratory Muhimbili National Hospital, Dar es Salaam

M Kasubi, MD, MMed (Microbiology and Immunology), PhD

Corresponding author: S Moyo (sabmoyo26@yahoo.com)

Enterobacteriaceae, oxacillin resistance in staphylococci, penicillin resistance in streptococci, and vancomycin resistance in enterococci (and eventually staphylococci).[2,4] The changing spectrum of microbial pathogens together with antimicrobial resistance trends and clinical significance necessitate continuous monitoring of antimicrobial resistance. Surveillance of antimicrobial resistance is critical in guiding physicians towards appropriate choice of antimicrobial agents to treat BSI. We aimed to describe the cauative agents and antimicrobial susceptibility patterns of bacterial isolates from patients with BSI.

Methods

The study was a retrospective analysis of blood cultures performed during the past 5 years (January 2005 - December 2009) at the Microbiology Section, Central Pathology Laboratory (CPL), Muhimbili National Hospital (MNH), Dar es Salaam, Tanzania. MNH is a tertiary health care facility handling patients with severe disease and those who require specialised management from three municipal hospitals in Dar es Salaam. All blood culture isolates that were detected and judged to be clinically significant, and antimicrobial drug susceptibility results of the bacteria isolated, were reviewed and included. Data including date of blood culture specimen collection, admitting hospital department (intensive care unit (ICU), paediatrics), blood culture and antimicrobial susceptibility results were recorded using a study data collection form.

Bacterial isolation, identification and antimicrobial susceptibility testing

Micro-organisms were isolated and identified according to a standard operating procedure.[5] Blood culture bottles received at the CPL were incubated aerobically at 37°C for 7 days. The bottles were examined daily for evidence of bacterial growth, haemolysis, gas production or turbidity above the red cell line. Subcultures using sterile syringes were done on blood agar, chocolate agar and MacConkey agar media daily for 7 days before blood cultures were reported as negative.[6] For antimicrobial susceptibility, disc diffusion tests were performed for all the isolates according to the method recommended by the Clinical and Laboratory Standard Institute.[7] Antibiotic disc (Oxoid) was applied to each plate, and after incubation at 35°C for 24 hours zone sizes were measured and interpreted accordingly.

Statistical analysis

Data were entered in Microsoft Excel 2000 (Microsoft Corp., Redmond, WA). The frequencies and proportions of bacteria isolated and antimicrobial susceptibility results were analysed. The data were also subjected to normality and homogeneity of variance tests. Where assumptions for parametric tests were not met, data were subjected to non-parametric tests and analysed using GraphPad Instant™ 1990 - 1993 software. A *p*-value of <0.05 was regarded as statistically significant.

Results

During the 5-year period, a total of 13 833 blood cultures were performed, 8 682 (62.8%) from children and 5 151 (37.2%) from adults. Bacterial pathogens were detected in 1 855 (13.4%) of the cultures; of these 48.8% (906) were from children and 51.2% (949) from adults. Positive blood cultures were detected in only 33 blood specimens from the ICU (1.8%) compared with 1 822 (98.2%) from other wards. Gram-positive bacteria (1 523; 82.1%) were significantly more prevalent (*p*=0.008) than Gram-negative bacteria (332; 17.9%) (Fig. 1).
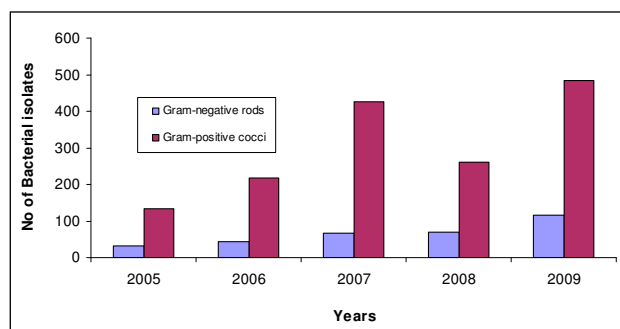

Fig. 1. Prevalence of bacteraemia due to Gram-negative rods and Gram-positive cocci from blood cultures at Muhimbili National Hospital, Dar es Salaam Tanzania, from 2005 to 2009.

The most common bacterial pathogens isolated were CoNS (1 250; 67.4%), *S. aureus* (245; 13.2%), *E. coli* (131; 7%) and *Klebsiella* spp. (130; 7.0%). Other bacterial pathogens detected were *Proteus* spp. (34; 1.8%), *Pseudomonas* spp. (22; 1.2%), *Streptococcus* spp. (28; 1.5%), *Salmonella* spp. (11; 0.6%), *Enterobacter* spp. (3; 0.2%) and *Acinetobacter* spp. 1 (0.1%) (Table I). Rates of detection of *E. coli* and *Proteus* spp. have been increasing over the years, while those of *Klebsiella* spp. and *S. aureus* have fluctuated in the past 5 years.

All bacteria isolated in our study showed a high level of resistance to the commonly used antimicrobial agents, including penicillin G (70.6%), tetracycline (63.8%), cefotaxime (62.5%) and ampicillin (62.3%). There were moderate to high levels of resistance to chloramphenicol (45.2%), erythromycin (35.0%), ciprofloxacin (29.3%), co-trimoxazole (25.0%) and gentamicin (23.5%). Gram-positive bacteria showed a very low level of resistance to vancomycin (1.5%), whereas *S. aureus* showed moderate level (23.3%) of resistance to methicillin (Table II).

Discussion

Clinically significant BSI is associated with high morbidity and mortality and considerably impacts on health care costs, especially when caused by antimicrobial-resistant bacteria. In our study 13.4%

Table I. Bacteria isolated from blood cultures at Muhimbili National Hospital, Dar es Salaam, Tanzania, from 2005 to 2009

	N	%
E. coli	131	7.1
Klebsiella spp.	130	7.0
Proteus spp.	34	1.8
Pseudomonas	22	1.2
Salmonella	11	0.6
Enterobacter	3	0.2
Acinetobacter	1	0.1
S. aureus	245	13.2
Streptococcus	28	1.5
CoNS	1 250	67.4
Total CP*	1 855	13.4‡
Total BL†	13 833	

*Total total cultures positive.
†Total blood cultures collected.
‡Rate of bacteraemia (CP/BL) × 100.

Table II. Antimicrobial resistance results of bacteria isolated from blood cultures at Muhimbili National Hospital, Dar es Salaam, from 2005 to 2009

	Gram-positive bacteria		Gram-negative bacteria		Total	
	N	%*	N	%*	N	%
Ampicillin	32	37.5	135	68.1	167	62.3
Gentamicin	50	26	214	22.9	264	23.5
Ciprofloxacin	158	42.4	154	15.6	312	29.3
Chloramphenicol	1	100	30	43.3	31	45.2
Erythromycin	189	34.4	14	42.9	203	35
Tetracycline	125	64.8	121	62.8	246	63.8
Penicillin G	174	68.9	13	92.3	187	70.6
Vancomycin†	135	1.5	-	-	135	1.5
Cefotaxime‡	-	-	8	62.5	8	62.5
Methicillin§	103	23.3	-	-	103	23.3
Co-trimoxazole	8	25	16	25	24	25

*Proportion of resistant isolates.
†Tested against Gram-positives only.
‡Tested against Gram-negatives only.
§Tested against *S. aureus* only.

of all blood cultures yielded positive results. The low rate may be explained by the fact that most patients probably received clinical care, including antimicrobial agents, at the municipal hospitals before admission to MNH, the referral hospital. Information on patients' clinical history before referral, including the use of antimicrobial agents, was not available.

Our study showed that Gram-positive bacteria (1 523; 82.1%) were significantly more prevalent than Gram-negative bacteria (332; 17.9%). These findings concur with those reported in previous studies in Tanzania and other African countries.[8,9] The current shift from Gram-negative to Gram-positive bacteria in causing BSI has been explained by others.[6] It has been suggested that this change is due to evolution of medical care, Gram-positive cocci having begun to re-emerge as predominant pathogens in BSI in the early 1980s.[10]

The most commonly isolated bacteria in this study were CoNS, followed by *S. aureus*, *E. coli* and *Klebsiella* spp. Our findings are consistent with the results of other surveillance studies.[9,11] CoNS are one of the most common causes of nosocomial BSI and also the most common blood culture contaminant.[11] Because only one blood culture was obtained for each of our study patients, it was not possible to determine whether patients who had CoNS isolated had a true bacteraemia or the finding was due to skin contamination. It is also possible that the recovery of CoNS could have resulted from other factors such as prolonged use of invasive intravascular devices, prolonged hospital stay, or other underlying co-morbidities. However, other previous studies identified CoNS (37%) as the leading pathogens in BSI.[10] The increased isolation of CoNS in blood cultures may also reflect a change from regarding these organisms as normal skin flora to viewing them as clinically significant. This is supported by the tremendous increase in the isolation of CoNS from 49 isolates in the year 2005 to 476 isolates in the year 2009. We therefore recommend that in our tertiary hospital guidelines should be provided to ensure that two sets of blood cultures are obtained to distinguish true CoNS bacteraemia from contamination. Hospital staff responsible for drawing blood for cultures should also receive training on aseptic technique.[12] *S. aureus* was the most common

pathogen detected, accounting for 13.2% of all positive blood cultures.

Paediatric wards accounted for 62.8% of blood cultures in this study, but few strains of *S. pneumoniae* were recovered and there was no recovery of *H. influenzae* from children. These pathogens are common causes of invasive bacterial infections in an unvaccinated paediatric population.[13-15] The World Health Organization (WHO) estimates that *H. influenzae* is the leading cause of bacterial meningitis in children under 5 years of age and the second leading cause of deaths due to bacterial pneumonia in resource-limited areas where vaccination is not performed.[16,17] However, *H. influenzae* type B vaccination was introduced in 2009 and could not have influenced the current findings. It is possible that laboratory-prepared culture media made with human blood might inhibit or not support the growth of strains of *H. influenzae*. A laboratory surveillance system for *S. pneumoniae* and *H. influenzae* is needed as an important first step to determine the prevalence and incidence of invasive pneumococcal and *H. influenzae* infections, and to measure the impact of the introduction of vaccination. As noted, measures to build laboratory capacity will ultimately improve diagnostic and surveillance capabilities.

Mortality and morbidity from BSI infections are greater when caused by antimicrobial-resistant bacteria, and proper treatment becomes a challenge.[18] We observed resistance to several antimicrobial agents used for first-line and inexpensive treatment of BSI, such as ampicillin, co-trimoxazole, penicillin and gentamicin. This concurs with findings of Aboud *et al.*, who reported resistance to first-line antibiotics among bacterial isolates from BSI in the same hospital.[8] We also documented high methicillin resistance among *S. aureus*; 23.3% of our bloodstream isolates were resistant to methicillin. The methicillin-resistant *S. aureus* (MRSA) rate found in this study is higher than that reported 10 years ago from the same hospital (0.4%), and relatively higher than reported 5 years ago (12%).[18,19] In Bugando referral hospital in northern Tanzania a prevalence of MRSA of 16.2% was reported,[20] implying that there has been an increasing trend in MRSA spread in the country, as is the case worldwide.

Although not investigated in our study, bacteraemia due to MRSA is associated with high mortality compared with methicillin-sensitive *S. aureus* bacteraemia.[9] The Enterobacteriaceae in this study demonstrated a high level of resistance (62.5%) towards third-generation cephalosporins. Third-generation cephalosporin resistance is usually caused by expression of extended-spectrum beta-lactamase (ESBL) enzymes, and this was probably the case among our strains, although specific confirmation of ESBL production was not performed. ESBL strains have been reported previously in septicaemic and ICU patients from the same hospital.[2,3]

Lack of enforcement of mechanisms regulating antibiotic use as a part of infection control programmes could have influenced the pattern of resistance results to a considerable degree. In addition, there are no guidelines to isolate hospitalised patients found to carry MRSA or ESBL producing strains. This situation in part explains the increased spreading of MRSA and ESBL strains observed in this study.

Conclusions

CoNS accounted for two-thirds of the bacterial pathogens isolated in our study. A high level of resistance to first-line and inexpensive antimicrobial agents was seen. Routine screening for ESBL production and methicillin resistance among Gram-negative rods and *S. aureus* from blood cultures should be instituted to monitor spread of multidrug-resistant isolates.

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