Bacteraemia in Malawian neonates and young infants 2002–2007: a retrospective audit

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ABSTRACT

Objectives: To assess the causes of bacteraemia in young infants and susceptibility to first-line antibiotics (benzylpenicillin plus gentamicin) at the Queen Elizabeth Central Hospital (QECH), Malawi during 2002–2007. Design: Retrospective analysis of demographic and microbiological data using laboratory records. Setting: QECH is Malawi’s largest hospital with 7000 neonates admitted annually, 9% for septicaemia. Patients: All infants aged 60 days or less admitted to QECH that had a blood culture taken over the 6-year period. Main outcome measures: 6754 blood cultures were taken. 3323 organisms were isolated: one-third were pathogens, two-thirds contaminants. Gram-positive organisms (53%) were more common than gram-negatives (47%). Four organisms made up half of all pathogens: Staphylococcus aureus (15.3%), group B streptococci (13.5%), non-typhoidal salmonellae (12.6%) and Escherichia coli (10.5%). Apart from non-typhoidal salmonellae and Streptococcus pneumoniae, most organisms were more common in the first week of life than later. Overall, 28% of isolates during 2002–2007 were resistant to first-line antibiotic, higher than observed during 1996–2001 (22%). Penicillin susceptibility fluctuated while gram-negative susceptibility gentamicin increased from 17% to 27% over the study period. Conclusions: In the QECH, pathogens causing young infant sepsis are an unusual mix of organisms seen in both developed and developing countries. Resistance to first-line antibiotics is higher than observed in most studies. Ongoing monitoring is needed and clinical outcome data would aid interpretation of findings. A high proportion of blood cultures were contaminated with skin flora—improved training and supervision of phlebotomists are needed to improve the utility of taking blood cultures.

INTRODUCTION

The Queen Elizabeth Central Hospital (QECH) in Blantyre, Malawi, is the country’s largest hospital and a referral centre for the southern region. Seven thousand neonates are admitted annually, including ~9% for septicaemia. Septic infants aged 60 days or less are treated with parenteral penicillin (50 000 units or 30 mg/kg three or four times a day). One of the largest single-site studies of young infant sepsis reviewing every blood culture sample taken for sepsis during a 6-year period (2002–2007).

ARTICLE SUMMARY

| Article focus |
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| Identification of bacterial causes of sepsis in young infants aged 2 months or less who presented to the QECH, Malawi during 2002–2007. |
| Documentation of antibiotic resistance patterns of bacterial pathogens isolated during this period. |
| Changes in aetiology and antibiotic resistance during this 6-year period compared with an earlier audit (1996–2001). |

| Key messages |
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| We found an unusual range of organisms infecting young infants, a range that lay between those classically observed in developed countries and those commonly reported in developing countries illustrating the complexities of local microbial ecology. |
| We documented a concerning level of resistance to empirical first-line antibiotics: 28% of isolates were resistant during 2002–2007 compared with 22% during 1996–2001. |
| The high number of horizontally acquired pathogens and the high proportion of contaminant organisms cultured highlight the need for greater attention to infection control practices. |

| Strengths and limitations of this study |
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| One of the largest single-site studies of young infant sepsis reviewing every blood culture sample taken for sepsis during a 6-year period (2002–2007). |
| Comparison with an earlier audit mapping trends in organisms and antibiotic resistance over a 12-year period. |
| Clinical outcome data were not linked to laboratory registers so the implications of the high level of antibiotic resistance are not immediately clear. |
| The high number of contaminants isolated may have obscured the growth of fastidious organisms. |

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We reviewed microbiological data from blood cultures taken from children in this age group during a 6-year period, 2002 to 2007, to assess further changes in the causes of bacterial infections and antibiotic resistance.

METHODS
We conducted a retrospective analysis of all blood cultures from children aged 60 days or less admitted to QECH during 2002–2007. We collected demographic and microbiological data from ward admission and laboratory registers and validated these against the electronic microbiology database. Case notes were not linked to these registers, so comorbid conditions (eg, malnutrition, HIV status, low birth weight), length of hospital stay and clinical outcomes could not be assessed. Data on bacterial colonisation of mothers from vaginal swabs and antibiotic treatment during delivery were not available.

Prior to 2006, routine blood cultures were collected on clinical suspicion of sepsis after malaria had been excluded. In order to save resources, from 2006, they were taken only if first-line antibiotic therapy had failed.

Samples were processed in the Malawi-Liverpool-Wellcome Trust Clinical Research Programme at QECH using British Society for Antimicrobial Chemotherapy guidelines to identify organisms and test for resistance to antibiotics. Isolates were classified as being either resistant or susceptible to an antibiotic using the disc diffusion method—minimum inhibitory concentrations were not routinely quantified.

Mixed skin flora and four specific organisms were considered contaminants and as such were excluded from the analysis: *Bacillus* sp, *Coagulase-negative staphylococcus*, *diphtheroids* and *Micrococcus* sp. Although *Coagu-

lase-negative staphylococcus* are recognised pathogens in intensive care settings, particularly for premature and low birth weight infants, we classified them as contaminants because care of sick infants at the QECH does not involve central venous access or other invasive care procedures.

EpiData 3.1 (EpiData Association) was used for data entry. Data were analysed by isolates, age group, year and resistance to antibiotics using Stata V.10.0 (StataCorp LP). Missing data are reported to aid interpretation of findings. In selected instances, we compared our data with findings from an earlier audit of blood (and cerebrospinal fluid) cultures in the QECH.

Ethical approval was not required for a retrospective audit of unlinked laboratory data.

RESULTS
Organisms isolated
Over the 6-year period, 6754 blood cultures were performed on infants aged 60 days or less. Fifty-five per cent (3726) were collected from inpatients of the main paediatric nursery that were mostly born outside the hospital; 40% (2673) were from infants in the special care baby unit that were born at the QECH or in another healthcare facility and transferred directly to this unit. Two hundred and sixty-three specimens were from children in other wards (3.9%) or the ward was not recorded (1.4%). The number of blood cultures collected increased annually until 2005, after which blood cultures were taken only in children who did not clinically improve on first-line therapy.

Forty-four per cent (2971) of blood cultures grew a single organism and 2.5% (168) grew two or more organisms (table 1). One-third (31%) of organisms were defined as clinically important pathogens, while two-thirds (69%) were contaminants. The proportion of contaminants was similar in different age groups. The proportion of blood cultures with contaminants,
Table 2: Comparison of neonatal blood culture audits: 1996–2001 versus 2002–2007

| Year of audit | Blood cultures | Proportion* of blood cultures with | Proportion of gram-positive: gram-negative pathogens |
|---------------|----------------|-----------------------------------|-----------------------------------------------------|
|               |                | No growth | Contaminants | Pathogen(s) |                                       |
| 1996–2001     | 2442           | 50% (1213) | 32% (781) | 18% (448) | 54%: 46% |
| 2002–2005*    | 5151           | 50% (2663) | 34% (1755) | 16% (809) | 55%: 45% |
| 2006–2007*    | 1603           | 60% (952)  | 30% (478)  | 11% (183) | 43%: 57% |
| 2002–2007*    | 6754           | 54% (3615) | 33% (2233) | 15% (992) | 53%: 47% |

*Cumulative proportions may exceed 100% during 2002–2007 as some cultures grew both pathogens and contaminants.

Table 3: Ten most common pathogenic bacterial isolates for all age groups, 2002–2007 (includes isolates of those infants whose age was unknown)

| Bacterial isolates                  | Proportion of pathogens in each year |
|-------------------------------------|--------------------------------------|
|                                     | 2002 (n=150) | 2003 (n=190) | 2004 (n=234) | 2005 (n=281) | 2006 (n=111) | 2007 (n=78) | 2002–2007 (n=1044) |
| **Gram positive**                   |                            |
| Staphylococcus aureus               | 19%                       | 19%          | 15%          | 16%          | 13%          | 4%          | 15.3%             |
| Streptococcus—group B (S agalactiae)| 15%                       | 13%          | 11%          | 18%          | 13%          | 6%          | 13.5%             |
| Streptococcus—alpha haemolytic      | 3%                        | 2%           | 11%          | 12%          | 5%           | 5%          | 7.4%              |
| Streptococcus pneumoniae            | 5%                        | 4%           | 6%           | 5%           | 6%           | 15%         | 5.9%              |
| Streptococcus—group D (Enterococcus)| 1%                        | 2%           | 4%           | 10%          | 3%           | 4%          | 4.4%              |
| Other gram positive                 | 7%                        | 5%           | 9%           | 4%           | 10%          | 0%          | 6.3%              |
| **Gram negative**                   |                            |
| Non-typhoidal Salmonellae sp*       | 23%                       | 14%          | 9%           | 7%           | 16%          | 18%         | 12.6%             |
| Escherichia coli                    | 7%                        | 13%          | 12%          | 7%           | 10%          | 19%         | 10.5%             |
| Klebsiella sp*                      | 6%                        | 9%           | 4%           | 6%           | 10%          | 14%         | 7.3%              |
| Enterobacter sp*                    | 2%                        | 5%           | 5%           | 5%           | 5%           | 4%          | 4.6%              |
| Acinetobacter sp*                   | 3%                        | 2%           | 2%           | 5%           | 2%           | 5%          | 3.1%              |
| Other gram negative                 | 10%                       | 12%          | 12%          | 6%           | 8%           | 5%          | 9.0%              |

Values in bold represent top three most common organisms in each year.

*Category is a combination of related organisms: Acinetobacter sp: A baumannii, A lwoffii and other Acinetobacter sp. Enterobacter sp: E agglomerans, E cloacae, E sakazakii and other Enterobacter sp. Klebsiella sp: K pneumonia, K oxytoca and other Klebsiella sp. Non-typhoidal Salmonellae sp: S typhimurium, S enteritidis and other Salmonella sp.

All pathogens were bacterial with the exception of a single yeast isolate (Candida sp) cultured in 2005. A greater proportion of gram-positive bacteria (53%; 551 isolates) were isolated than gram-negative bacteria (47%; 493 isolates). The change in policy in 2006 to collect blood cultures only from children who failed empiric therapy led to a number of changes: there was a reduction in the proportion of pathogens grown—11.4% of all cultures had at least one pathogen in 2006–2007 compared with 15.7% in 2002–2005 (OR=0.7, 95% CI 0.7 to 0.8, p=0.000); and gram-negative organisms became more common in 2006–2007 compared with 2002–2005 (OR=1.6, 95% CI 1.2 to 2.2, p=0.003). The 10 most common organisms isolated constituted 85% of all cultured pathogens (table 3). Four organisms made up over half of all pathogens isolated: Staphylococcus aureus (15.3%), group B streptococci (GBS) (13.5%), non-typhoidal salmonellae species (NTS) (12.6%) and Escherichia coli (10.5%). These four organisms were the four most common isolates for 3 of the 6 years under review and made up a significant proportion of isolates in each age group: 0–7 days (47%), 8–28 days (66%) and 29–60 days (51%). With the exception of NTS and Streptococcus pneumoniae, most organisms were more common in the first week of life than later (figures 1 and 2).

Antibiotic susceptibility

More than 25% of tested isolates was resistant to both penicillin and gentamicin in 2003, 2005, 2006 and 2007, with 28% resistant for the combined 6-year period. Organisms were more likely to be resistant to first-line therapy in 2007 than 2002 (OR=2.2, 95% CI 1.1 to 4.3, p=0.024); however, there was no difference for the period 2002–2005 when cultures were collected routinely for septicaemia compared with 2006–2007.
when cultures were collected after first-line treatment had failed (OR = 1.0, 95% CI 0.7 to 1.5, p = 0.86). For common organisms (NTS, E coli, Klebsiella sp, S aureus), the proportion susceptible to first-line antibiotics in 2006–2007 was similar to or greater than in 2002–2005.

Susceptibility to penicillin fluctuated annually for all isolates, while the resistance of gram-negative organisms to gentamicin—the main antibiotic used to treat gram-negative sepsis—increased from 17% in 2002 to almost 27% in 2007 (OR = 1.8, 95% CI 0.8 to 4.2, p = 0.18) (tables 4 and 5). A small number of both gram-negative and gram-positive bacteria were resistant to ceftriaxone, the second-line antibiotic therapy (including a single S pneumoniae isolate that showed borderline intermediate resistance to ceftriaxone (zone = 26 mm, sensitive = 27 mm, resistant = 22 mm)). Most gram-negative isolates were susceptible to ciprofloxacin throughout the study period (tables 4 and 5), whereas gram-negative resistance to chloramphenicol, tetracycline and co-trimoxazole remained consistently high for all common organisms (table 5). The high proportion of gram-positive organisms resistant to penicillin (47% for 2002–2007) (table 4) reflects the frequency of S aureus infections, of which 94% of isolates were penicillin resistant. Of the staphylococci tested (32% of total), only 2% were resistant to cloxacillin.

**DISCUSSION**

**Isolated organisms**

In our study, a clinically important bacterial pathogen was isolated from 15% (992) of young infant blood specimens. The proportion of pathogens that were gram positive (53%) and gram negative (47%) was similar to the previous audit from 1996 to 2001 in the same hospital. Our observations among this cohort differ from those reported elsewhere in Africa, which describe a preponderance of gram-negative bacteria. The range of organisms found in our study fits somewhere between those classically observed in developed countries—such as GBS and S aureus—and those more commonly reported from developing countries including Klebsiella, Acinetobacter and Pseudomonas. The four most common
pathogens were *S. aureus*, GBS, NTS and *E. coli*. Similar findings from earlier studies in the French Antilles and South Africa, particularly with regard to the frequency of GBS, led some researchers to speculate about an epidemiological transition occurring in middle-income countries of bacteria infecting neonates or an inherent bias in reporting with many neonates born at home dying before reaching hospital. Both suppositions seem unlikely for the QECH: the United Nations ranks Malawi in the lowest category for development, and the type of organisms we isolated have remained unchanged since 1996, while most (79%) mothers deliver in health facilities in this part of Malawi. The complex local microbial ecology may partly explain the range of organisms seen in the QECH.

Undernourished children (10% of paediatric inpatients in the QECH) are more likely than well-nourished children to have bacteraemia on admission and to be infected with gram-negative organisms. Gram-negative sepsis due to NTS, is associated with the wet (malaria) season in both The Gambia and Malawi and may explain the predominance of NTS in our study compared with reports from other developing countries where *Klebsiella* spp and *E. coli* are more common. NTS infection has also been shown to be common among adults with HIV in Malawi with a high recurrence rate after treatment.

Recent data from Zimbabwe found no difference in GBS colonisation rates between women with and without HIV-1 infection. Studies within Malawi demonstrated that GBS with type 3-polysaccharide antigen was common and more virulent than other serotypes. In contrast, in the Gambia, maternal colonisation with GBS type 3 is infrequent (6%) and neonatal GBS sepsis is uncommon.

*S. pneumoniae* was a leading cause of sepsis in older infants aged 28–60 days, a finding consistent with other local and international studies.

The pattern of pathogens reflects the fact that neonates admitted to our wards are from both home and health facility deliveries. The 2004 national demographic and health survey showed that of all deliveries in the southern region of Malawi, the catchment area for the QECH, 79% were in health facilities. We were not able to track the place or time of birth in relation to the time of hospital admission in our study. Therefore, we cannot separate community-acquired infections from those acquired in health facilities or maternally spread organisms from those transmitted by unhygienic practices in health facilities or at home. Nonetheless, the prevalence of gram-negative and *S. aureus* infections in young infants of all ages suggests that hospital-acquired infections are important, and the high proportion of contaminants isolated likely reflects a general lack of infection control practices in the hospital and the need for improved training.

The 2006 policy to take blood cultures only when first-line therapy failed led to relatively fewer pathogens

### Table 4

| Antibiotic          | Proportion of isolates susceptible (number tested) |
|---------------------|--------------------------------------------------|
|                     | 2002  | 2003  | 2004  | 2005  | 2006  | 2007  | 2002–2007 |
| Penicillin          | 57% (74) | 42% (85) | 55% (106) | 55% (149) | 47% (51) | 62% (26) | 53% (491) |
| Erythromycin        | 86% (74) | 81% (86) | 69% (108) | 67% (163) | 81% (52) | 78% (27) | 75% (510) |
| Chloramphenicol     | 84% (74) | 84% (86) | 77% (109) | 73% (167) | 77% (53) | 74% (27) | 78% (516) |
| Co-trimoxazole      | 40% (75) | 45% (84) | 46% (105) | 47% (165) | 40% (53) | 22% (27) | 43% (509) |
| Gentamicin          | 55% (49) | 100% (16) | 77% (35) | 73% (49) | 93% (14) | 100% (3) | 73% (166) |
| Ceftriaxone         | 94% (18) | 91% (74) | 93% (69) | 94% (109) | 97% (36) | 87% (23) | 93% (329) |
| Tetracycline        | 40% (75) | 47% (86) | 31% (110) | 35% (165) | 38% (53) | 52% (27) | 38% (516) |
| First-line therapy  | 88% (75) | 60% (86) | 78% (107) | 74% (155) | 71% (51) | 73% (26) | 74% (500) |

(Proportion of strains tested: 551)
culturated overall. This is likely a function of selecting children whose symptoms were due to fastidious organisms and non-bacterial causes as well as those whose clinical improvement lagged behind their microbiological improvement (ie, they still had symptoms but no bacteraemia). The policy change also led to more gram-negative isolates that may reflect a reduced susceptibility of these organisms to first-line antibiotics as was seen in 2007 compared with 2002.

Antibiotic resistance and implications for empirical first-line antibiotic regimens

In this study, almost three in 10 isolates were resistant to first-line antimicrobial therapy. This is slightly higher than was recorded in the previous audit and higher than has been observed in much of the literature.2 It is also important to note the worrying shift towards resistance to ceftriaxone, which had not been present earlier and has emerged with the increasing use of ceftriaxone as second-line therapy. Streptococcal species resistance is of particular concern as there is no locally available third-line antibiotic therapy.

As in other developing countries, we found the appearance of multidrug-resistant gram-negative organisms in particular Klebsiellae.4 Resistance of E coli to gentamicin rose from 7% during 1996–2001 to 20% during 2002–2007. Unfortunately, we lacked clinical data to compare in vitro and in vivo resistance for these pathogens, which differed in past assessments in the QECH.5

During the 6-year period of our study, there was a 10% increase in resistance to chloramphenicol for gram-positive pathogens possibly reflecting the widespread use of this agent for first-line treatment of sepsis, especially in malnourished children. Few isolates of S aureus were resistant to cloxacillin, a pattern seen elsewhere in the developing world,10 and most remain susceptible to gentamicin.

Although this is a large data set and the level of resistance to first-line treatment of isolated pathogens is high and rightly warrants concern, the implications for antibiotic therapy are not straightforward. On the one hand, 28% of pathogens were resistant to first-line antibiotics. Some laboratory-based surveillance systems recommend revising treatment when more than 5% of isolates have in vitro resistance to a particular antibiotic.19 On the other hand, of all blood culture specimens taken just one in 25 grew a resistant organism. Interestingly, there was no difference in the level of resistance to first-line therapy before and after the 2006 change in policy for the indication of taking blood cultures.

Limitations

The pathogens isolated may not reflect the causative organisms for young infant sepsis in rural Malawi for a number of reasons: deaths of newborns outside the hospital were not captured,20 the number of isolates with unknown ages could have affected the distribution of organisms across the age categories, the high number of contaminants isolated may have obscured the detection of more fastidious pathogens and isolates taken after the change in policy for taking blood cultures may reflect organisms that grow despite antibiotic exposure. The policy change may also have affected sensitivity patterns when compared with previous studies. Length of stay, which has been associated with nosocomial spread of pathogens with higher levels of antibiotic resistance, was not recorded in our data set.

CONCLUSIONS

This study is one of the largest single-site studies of its kind in developing countries and highlights the importance of local aetiological assessments of young infant sepsis for review of empiric treatment. Arguably, such assessments are becoming more important as standard treatment recommendations from international bodies are increasingly applied. Audits should ideally be correlated with clinical outcomes and separate community-acquired from hospital-acquired infections to determine the most appropriate first-line antibiotic regimens. This would also facilitate consideration of other preventive measures such as vaccination and screening. For example, the high prevalence of GBS sepsis in Malawi (combined with a high case death rate (33%)) warrants routine screening of mothers with antibiotic prophylaxis and maternal immunisation.16 The high proportion of blood cultures contaminated by skin flora in both this and the previous audit shows an urgent need for additional training and supervision. Other studies of children have recorded lower contaminant rates of 13%–23%.21 22 Trained phlebotomists and a formal internal system of monitoring and quality assurance should be considered.23

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