Emergence of Multidrug-resistant Gram-negative Organisms in a Neonatal Unit and the Therapeutic Implications

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Summary

Multidrug-resistant organisms are increasing worldwide. Over the years we have noted increasing resistance of organisms isolated in our neonatal unit. There is a need therefore to scrutinise the problem so as to be able to plan for the future. Over a 5-month period, 716 infants were admitted of which 192 were screened for sepsis. Overall, 121 (16.7 per cent) had positive blood cultures. The predominant organisms were Gram negative (73.6 per cent of isolates) with *Klebsiella* species topping the list at 31 per cent. Case fatality for infants infected with Gram negative organisms was 41 per cent. Resistance to gentamicin was 20 per cent chloramphenicol 23.6 per cent, and amoxicillin/ampicillin 66.3 per cent. Of worry is the resistance to ceftazidime 19.1 per cent, and cefuroxime 21.3 per cent, with the figures rising to 27 per cent when more specialized tests are done (disc approximation and potentiation tests). If these drugs cannot be used in 20–27 per cent of cases then the situation is serious. The contributory factors to increased resistance include: non-investigation of infants put on antibiotics (50 per cent of cases); prolonged (73 per cent) and sometimes unjustified (41.7 per cent) use of antibiotics; and non-utilization of investigations when these are done (52 per cent) together with the delay in getting results back in the ward (6 days).

Introduction

Diagnosis of infection in the neonate depends very much on a high index of suspicion and appropriate investigations. All sick neonates are generally presumed to be infected until proven otherwise. If all is working well, one should be able to tell which baby is infected within 72 h. Without investigations and/or results there is a tendency for indiscriminate, prolonged and inappropriate use of antibiotics. This very soon leads to multidrug-resistant organisms.

The production of β-lactamases is the primary cause of resistance to the β-lactam group of antibiotics, which is one of the most rapidly developing and clinically significant antimicrobial-resistant mechanisms. The recent emergence of Gram-negative pathogens capable of hydrolysing the new expanded spectrum cephalosporins (ESCs), either by plasmid- or chromosomally-mediated enzymes, is not easily recognized by most of the routine susceptibility tests in the bacteriology laboratory. Failure to identify these pathogens in high risk hospital units may lead to serious therapeutic failure with ESCs. In order to become aware of this problem laboratories need to employ additional specific tests to detect such pathogens by a routine surveillance.

Although we have not instituted continuous surveillance, we have noted that over the years the same organisms occur in general, namely *Escherichia coli*, *Klebsiella* species and *Citrobacter* species in the Gram-negative group, and *Staphylococcus aureus* and *S. epidermidis* in the Gram-positive group.[1–3] Antibiotic resistance has been noted to be higher in the early 1990s than 1980s. The present report concentrates on Gram-negative enteric organisms and aims to document the rate of infection, infant outcomes, the extent of plasmid-mediated and inducible β-lactamase production, as well as some contributing factors.

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Patients and Methods

Study site

Kenyatta National Hospital (KNH) Newborn Unit (NBU) was designed to cater for a maximum of 40 infants. But the monthly admissions average around 150 infants. With the inevitable long stay of very low birth weight infants, the bed occupancy is more than 100 per cent. Consequently two or three infants share a cot or an incubator. With this setting, the risk of cross infection is high. There are no intensive care facilities.

Clinical information

The study is partly retrospective and partly prospective.

For the retrospective period December 1997 to April 1998 (5 months) we used the laboratory reports for all infants investigated during that period noting those who had positive blood cultures. Using these reports we looked at the case records noting birth weight, age at investigation, duration of stay, outcome, interval between investigation and getting results back, and antibiotic use. For the prospective period during June 1998, all infants on antibiotics were scrutinized and also the total amount of antibiotics used.

For antibiotic use, the name of the antibiotic and the duration of therapy was recorded. An effort was made to determine the appropriateness/justification of antibiotic use by scrutinizing whether/if: (a) the clinical condition and progression justified the start and continued use of the antibiotic; (b) the clinical evaluation was satisfactory; (c) there were other conditions that may mimic sepsis; (d) the change of antibiotics was based on laboratory results; and (e) there was supportive management. Supportive management was deemed to be poor if the infants were allowed to become dehydrated, anaemia was not corrected in time, or nutrition issues were not addressed.

Methods

All clinical specimens were processed and isolates were identified by standard bacteriological techniques.[4] Antimicrobial susceptibility was performed by NCCLS disc diffusion method[5] which is routinely followed in the laboratory. Our laboratory is a participant in the international external quality assessment scheme of the World Health Organization collaborating centre for clinical microbiology at Leuven, Belgium.

Additional tests for ESC resistance were performed with all Gram-negative isolates showing susceptibility to ceftazidime, cefuroxime, cefoperazone.

Special tests for the detection of false susceptibility to ESCs in enteric bacilli were done as follows.

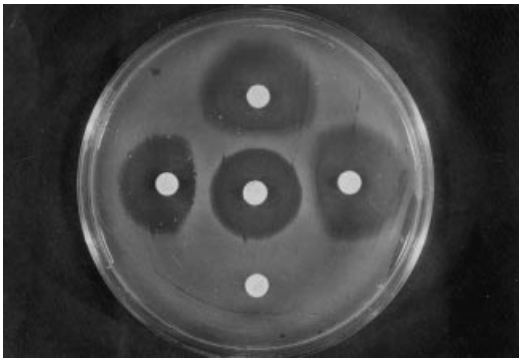

FIG. 1. Positive disc approximation test with an isolate of *Enterobacter* spp producing a flower pattern of flattened inhibition zones around discs of ESCs. Imipenem disc is in the centre.

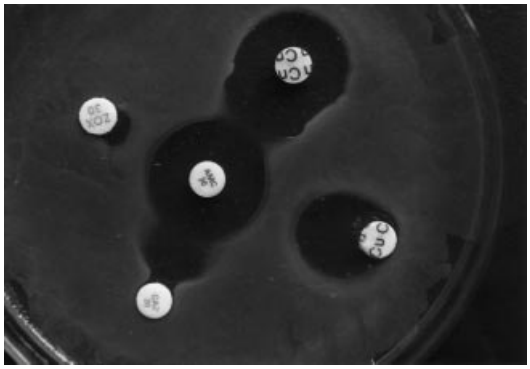

FIG. 2. Positive disc potentiation test showing amoxicillin/clavulanate disc in the centre and the ESCs surrounding it.

1. Disc approximation test[6] (Fig. 1). Diagnostic sensitivity test agar plates were inoculated with the test strain in the routine manner. A cefoxitin or imipenem disc is placed in the centre at 2.5 cm (centre to centre) from the ESC discs, such as cefuroxime, cefotaxime, or ceftazidime. After overnight incubation, a flattening of the zone of inhibition of the ESC disc towards the inducer disc by $\geq$1 mm indicated the presence of inducible β lactamase. Such isolates have to be reported resistant to all cephalosporins.
2. Disc potentiation test or double-disc synergy test for the detection of clavulanate-sensitive plasmid-mediated β lactamases[7] (Fig. 2). In this test a plate of diagnostic sensitivity agar is inoculated with the test organism in the routine manner. Discs of ESCs are placed 30 mm (centre to centre) from an amoxacillin–clavulanate disc and incubated overnight. Enhancement of the zone of inhibition of the ESC discs or the appearance of the so called ghost zone between the ESC discs and the clavulanate-containing disc, indicate the presence of plasmid-mediated β lactamase.

TABLE 1
Infant characteristics

	Alive ($n = 41$)	Died ($n = 28$)
Birthweight (g)		
Mean	2180	1477
< 2000	17	23
≥2000	7	1
≥2500	15	3
Not recorded	3	1
Duration of stay (days)		
Mean	23.5	18.3
Range	6–47 (120)	7–14

TABLE 2
Age at onset and management

	Alive ($n = 37$)	Died ($n = 11$)
Age at onset		
≤3 days	18	3
> 3 days	13	7
Not clear	6	1
Duration to get results		
No comment	18	7
Mean days	6	5
Duration of antibiotic use (days)		
Mean	16	13
Range	5–33	7–33
Poor support	15	8

All the above tests were carried out in duplicate to check for reproducibility and all negative tests were repeated with reduced disc spacing of 20–25 mm to confirm the results.

Limitations

Since most of the study is retrospective, several babies who were septic could have missed having a blood culture taken. We had no control over how the specimens were taken.

Results

The period of retrospective review was December 1997 to April 1998. Of the 89 laboratory reports produced, three were repeat specimens and six were neonates from other wards in the hospital. Hence analysis was for 80 infants who had been admitted to the NBU. For this group of infants it was possible to trace 48 (60 per cent) of the files, 37 of whom were alive and 11 had died. However, using the NBU admission book plus the traced files it was possible to obtain birth weight, and outcome for 69 (86 per cent) infants; out of these 41 were alive and discharged, while 28 had died.

Total admissions during the 5-month period was 716 infants, of whom 161 (22.5 per cent) died. The overall sepsis rate during this period was 16.9 per cent of which Gram-negative sepsis rate was 11 per cent. Case fatality was 41 per cent representing 18 per cent of all deaths in the period.

Table 1 shows the analysis of the 69 infants. Table 2 gives the analysis from the available files. It is noted that on the whole those who died had very low birth weight (VLBW). In two of the larger infants, death was related to congenital malformations and in the case of the second, to severe birth asphyxia rather than infection. Mean duration of stay in the two groups was similar. Two infants who stayed for 61 and 120 days are not included in the mean calculation. Prolonged stay for these two infants was related to the death of their mothers.

The age of onset is equally distributed between early and late onset (20 in each group). For 25 (52 per cent) infants there were no comments on culture results in the files and for those where it was done, it took 4–10 days to do so. Of the survivors, 15 (40.5 per cent) were on antibiotics for more than 2 weeks and eight (21.6 per cent) for more than 3 weeks. For the group that died, three were on antibiotics for more than 2 weeks and one infant was on antibiotics for slightly over 3 weeks. In total, 27 (73 per cent) with available information were on antibiotics for more than 2 weeks.

Antibiotic combinations were fairly standard.

TABLE 3
Antibiotics use per month (1998)

	Concentration/vial	Total used
Gentamicin	20 mg	400
Crystallin penicillin	1 mega unit	100
Ceftazidime	2 g	40
Amikacin	100 mg	140
Piperacillin	2 g	40
Ceftrioxone		10
Cloxacillin		10
Chloramphenicol		10

TABLE 4
Organisms isolated

Total number of blood cultures	192
Total number of positive cultures	121
Gram-negative bacilli (Total)	89
Klebsiella species	38
Citrobacter species	26
Enterobacter species	19
Salmonella typhimurium	2
Escherichia coli	4
Gram-positive cocci (Total)	32
Staphylococcus aureus	9
Staphylococcus epidermidis	19
Enterococci	4

TABLE 5
Antibiotic resistance of Gram-negative isolates

Antibiotic	Numbers resistant	Resistance implied by special tests
Amoxicillin/Ampicillin	59 (66.3%)	—
Ceftazidime	17 (19.1%)	24 (27%)
Cefuroxime	19 (21.3%)	24 (27%)
Amoxicillin/Clavulanate	12 (13.5%)	45 (50%)
Gentamicin	18 (20.2%)	—
Chloramphenicol	21 (23.6%)	—
Cotrimoxazole	20 (22.5%)	—

Crystalline penicillin and gentamycin were the first line in all cases and these were changed to amikacin/ceftazidime in most cases or amikacin/ceftrioxone in a few cases. For the 84 available episodes of antibiotic use, 48 (58.3 per cent) were judged as appropriate, 24 (28.6 per cent) unjustified and 11 (13.1 per cent) uncertain. The uncertainty arose from inadequate information in the notes.

Poor supportive management was evident in 23 (48 per cent) cases. Most of these were due to problems of fluid management in the VLBW infants, the majority of whom had feed intolerance.

For the month of June 1998, 54 infants were on antibiotics of whom 27 (50 per cent) were not investigated. For those who were investigated only six (22 per cent) had the results noted in their case files.

Table 3 shows the amount of antibiotics used per month during 1998. Assuming two doses per day with the most commonly used drugs and a 10-day course, 40–50 infants would have been on antibiotics per month. This is approximately one-third of total admissions.

Table 4 shows the organisms grown during the period of study. A total of 63 per cent of blood cultures taken were positive and of these 73.6 per cent were Gram-negative organisms. *Klebsiella* species still tops the list at 31 per cent of septicaemias. The antibiotic resistance pattern is shown in Table 5.

Twenty-one *Citrobacter* species and all *Enterobacter* species (19) gave a positive disc approximation test. Out of the 42 isolates of *Klebsiella* species and *E. coli* put together, 19 gave a positive disc potentiation test while six *Klebsiella* species were positive for the disc approximation test. Nine isolates (five *Enterobacter* species, two *Citrobacter* species and two *Klebsiella* species) were positive in both tests. Independent amoxicillin–clavulanate resistance was detected in four *Klebsiella* and two *E. coli* isolates.

Discussion

The overall sepsis rate of 16.9 per cent of all admissions has not changed in the past 15 years and is much higher than that reported by Khadilkar,[8] who gave a figure of 7.4 per cent, but fall within the range quoted by Donowitz.[9] Were these infants really infected or were these contaminants, considering that we had no control over how blood was taken? We believe that these were true infections, first, because many of these infants were seriously sick and second, because it is unusual to get Gram-negative bacilli organisms as contaminants. This is in contrast to Gram-positive cocci, especially *Staphylococcus epidermidis*. Although we report a rate of 16.9 per cent this is not a true prevalence because it is noted that up to 50 per cent of infants were treated without investigations. Failure to investigate was partly due to lack of specimen bottles but also due to the fact that clinicians did not consider investigation often. The fact that some septic infants might have missed being investigated suggests that our infection rate might be even higher.

The great majority of infants had Gram-negative sepsis, i.e. 10 per cent of all infants admitted. The case fatality rate of 41 per cent in this group is disturbing. It is noted that some of the deaths were due to inadequate support and observations. Intravenous fluids were not running well leading to dehydration. This was especially so in the VLBW infants. This finding is not surprising. Most sick VLBW infants did not tolerate feed for long periods and in a setting with inadequate facilities fluid management can be a nightmare. Starvation could have been an additional factor since adequate facilities for parenteral nutrition were lacking.

We did not have a matched control for a comparison of duration of stay. Other studies have shown a significant prolonged stay for infected infants.[10] In some of these studies prolongation has been related to birth weight rather than infection per se.[11] The mean weight of survivors was 2180 g. These are infants who came to the NBU with short-lived problems, but stayed for a mean of 23.5 days. We believe that the longer stay was because of infections.

A positive blood culture is the gold standard in the diagnosis of neonatal septicaemia. Additional investigations augment clinical features to make sepsis plausible. But we note that 50 per cent of neonates were on antibiotics without investigations. Even in those instances where investigations were done, in 52 per cent of cases they were not effectively utilized. In the absence of proof of sepsis many clinicians felt obliged to continue antibiotics for a full 10-day course. If the infant

is not infected he/she is being subjected to unnecessary treatment. There is also the danger of removing useful and/or susceptible micro-organisms and encouraging resistant ones. If this occurs then one is forced to go on to use more expensive antibiotics. Survival of the affected infant is also compromised. Since most of the study is retrospective, it is not possible to know if we would have altered the mortality by revising antibiotics, as was found in an earlier study.[1]

In the present study, antibiotic use extended beyond 14 days in 73 per cent of infants. The practice of prolonged antibiotic use without proof of infection is unjustifiable and in many cases questionable. We found that in 28.6 per cent of infants, antibiotic use was unjustified and in 13.1 per cent it was uncertain. This indicates inadequate clinical and laboratory evaluation of suspected sepsis. Besides creation of multidrug-resistant organisms, there is also the danger of drug toxicity especially with aminoglycosides. In our case infants were almost always on a combination of an aminoglycoside and a penicillin or a cephalosporin.

If an infant is on an antibiotic for more than a week without response, the clinician should be asking why. The questions which should be asked at this stage are: is the infant actually infected, are there resistant organisms, or is the supportive management at fault? In the period of review, repeat cultures were done in only three neonates. None of the reports indicate change of therapy on account of investigation.

A positive disc approximation test (30 organisms) indicates a presence of chromosomally mediated inducible β-lactamase which is insensitive to clavulanate. Since this enzyme is induced *in vitro* and is even more rapidly induced *in vivo*, β-lactam therapy is not likely to succeed. The effective drugs for these pathogens would then be cefoxitin, cefotetan, cefmetazole, and to some extent carbapenems. On the other hand, the positive disc potentiation test implies plasmid-mediated enzymes. These organisms can be treated with amoxicillin/clavulanate combinations while those producing chromosomal induced enzymes cannot be treated with the same antibiotic. The isolates which were positive in both tests have even more serious therapeutic implications. It is popularly believed by practitioners that a amoxicillin/clavulanate combination can treat almost any infection. But the extensive array of plasmid and chromosomal mediated β-lactamases with their different substrate affinity and different patterns of inhibitor sensitivities make it very difficult for any inhibitor drug combinations to be active against all these pathogens.

It should be noted that β-lactam resistance is not easily detected by routine laboratory susceptibility tests. In this case additional tests must be employed. These special tests are not currently available especially in smaller hospitals in the developing world. However, it is desirable that clinical microbiology laboratories incorporate these simple and inexpensive tests into routine practice.

The degree of multidrug resistance shown in this review has worrying therapeutic implications especially when one considers that cephalosporins have not been used for a long time in our neonatal unit. For the infants who pick up these organisms and develop sepsis, we shall reach a stage when we have no drug combination to treat them. On the other hand, it would be disastrous if these organisms were to spread to the community through mothers and their infants when they are discharged, or through the personnel working in the NBU. The time tested practice of strict infection control in high risk hospital units, combined with judicious antibiotic policy guided by laboratory testing of isolates is the only solution to this problem. These policy guidelines have to be strictly adhered to by everybody. It should be noted, however, that multidrug resistance is an increasing problem worldwide but perhaps more common in the developing world where access to a laboratory is limited. A recent review of the problem in East and Central Africa is available,[9] and Levy and Stephenson give some global views on the subject.[10,11]

This report shows that neonatal sepsis is endemic in our institution with high morbidity and mortality. The high rate of infection is due to overcrowding necessitated by a high admission rate and prolonged stay. It is also facilitated by poor infection control measures as was noted by the infection control nurse during the prospective period. The identified practices that could have encouraged the emergence of multidrug-resistant organisms include lack of adequate investigation, and prolonged and sometimes unjustified use of antibiotics. We plan to carry out continuous surveillance and regular staff education on infection management and control.

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