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Emergence of Multidrug Resistant Bacteria in Neonatal Infections: Implication for Institutional Antibiotic Formularies

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Abstract

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Background: Multidrug resistant bacteria are not uncommon in neonatal infections, hence the need to monitor the resistance pattern of offending organisms and periodically modify the empirical treatment protocol.

Objective: To determine the magnitude of multidrug resistant pathogens in newborns with systemic bacterial infections.

Patients and Methods: Records of neonates treated for septicaemia in the Special Baby Care Unit of Ahmadu Bello University Teaching Hospital, Zaria, from April 1, 2004 to March 31, 2008 were retrieved and results of microbiological studies extracted. Data were analyzed with Epi Info version 6 software. Statistical significance was set at $p < 0.05$.

Results: Of the 575 neonates evaluated for septicaemia, 123 had 200 bacteria isolated from blood and other sites. Predominant isolates were *Staphylococcus aureus* (50.0 percent), Klebsiella species (18.0 percent) and *Escherichia coli* (14.5 percent). Multidrug-resistance was seen with *Staphylococcus aureus* (3.0 percent), *Escherichia coli* (3.4 percent), Proteus species (11.1 percent) and Klebsiella species (22.2 percent).

Conclusions: The resistance pattern suggests the need to modify the empirical treatment protocol as follows: erythromycin for superficial infections, and a combination of cloxacillin and gentamicin for deep-seated infections. However, in severe deep-seated infections and meningitis, cefuroxime and ceftriaxone respectively, should be used in place of cloxacillin. Further studies are needed to identify the most appropriate antibiotics for treating multidrug resistant bacteria.

Key words: Bacterial agents, neonates, septicaemia, antibiotics resistance

Introduction

EVER since Fleming[1] identified the first case of resistance to penicillin, there has been growing concern over the increasing prevalence of antibiotic resistant pathogens and recently, of multidrug resistant agents in neonatal infections.[2,3] Although the cost of antibiotic resistance in patient management is difficult to compute, the accruing prolonged hospital stay, increased opportunity for pathogen transmission and of course, the high risk of mortality call for vigilance. The changing antibiotic susceptibility pattern is an indication for continuous monitoring of the resistance trends and modification of institutional antibiotic formularies.

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Patients and Methods

This retrospective study was conducted in the Special Care Baby Unit (SCBU) of Ahmadu Bello University Teaching Hospital, Zaria, and covered the period, April 1, 2004 to March 31, 2008. The hospital is a tertiary health centre that serves as a paediatric referral centre for Kaduna, Kaduna and Zamfara states and parts of Niger, Nassarawa, Kano, Plateau, Jigawa and the Federal Capital Territory. The newborn unit admits about 700 babies annually[4] and has two separate sections, one for in-born and the other for out-born babies. Medical records of neonates treated for septicaemia were retrieved and results of microbiological studies such as blood, urine, stool and cerebrospinal fluid (CSF) cultures as well

as cultures of swabs of body discharges were extracted.

Laboratory methods: Standard bacteriological techniques[3] were routinely used in the microbiology department of the hospital to process specimens and identify isolated pathogens. Antibiotic susceptibility testing of the isolates was performed using Kirby-Bauer disc diffusion technique.[5]

Statistical methods: The data obtained were entered into Epi Info version 6 and analyzed. Frequency tables were generated while the differences between proportions were tested using Chi square test with Yate's correction. Statistical significance was set at $p < 0.05$.

Results

Of the 575 neonates evaluated for septicaemia, 123 had positive blood cultures. Samples taken from other sites concurrently yielded positive growths in 21 CSF cultures, 17 urine specimens, 14 eye swabs, 11 umbilical swabs, 11 skin swabs and three ear swabs. The total number of isolates from the 123 septicaemic babies was 200. The distribution of isolates by the site of isolation is shown in Table I. Gram-positive bacteria were the predominant isolates (55.5 percent), of which 90.1 percent was *Staphylococcus aureus*. Gram-negative agents accounted for 44.5 percent of the isolates. Klebsiella species (18 percent) and *Escherichia coli* (14.5 percent) were the predominant gram-negative bacteria isolated.

The patterns of antibiotic resistance are displayed in Tables II and III, which show high resistance to commonly used antibiotics and zero resistance to ofloxacin and ciprofloxacin. *Staphylococcus aureus*, while multiplyresistant to penicillin, ampicillin, amoxicillin, co-amoxiclav and cotrimoxazole, was 27 percent and 34 percent resistant to erythromycin and cloxacillin, respectively. However, three strains (3.0 percent) of *Staphylococcus aureus* were resistant to all the antibiotics tested except to ofloxacin and ciprofloxacin. Streptococcus species were similarly resistant to the penicillins. The overall resistance to cloxacillin among the gram-positive bacteria was higher than resistance to gentamicin, even though the differences in proportion of resistances did not reach levels of statistical significance ($X^2 = 3.65$, df=1, $p = 0.056$). Similarly, erythromycin recorded lower rates of resistance than the combination of ampicillin and cloxacillin ($X^2 = 7.91$, df=1, $p = 0.005$). There was however no significant difference in the resistance rates between erythromycin and gentamicin ($X^2 = 3.15$, df=1, $p = 0.076$), erythromycin and cefuroxime ($X^2 = 0.18$, df=1, $p = 0.673$) and gentamicin and ceftriaxone ($X^2 = 2.05$, df=1, $p = 0.152$) among the gram-positive isolates.

The resistance pattern among the gram-negative bacteria was similar but generally higher than that of the gram-positive agents. Overall, resistance to the penicillin group of antibiotics was higher than resistance to the cephalosporin group ($X^2 = 29.50$, df=1, $p = 0.000$); there was however, no significant

Table I

Bacterial Isolates and Sites of Isolation

	Sites of Isolation							
Isolates	*Blood*	*CSF*	*Urine*	*Eye*	*Ear*	*Umbilicus*	*Skin*	*Total (%)*
Staphylococcus aureus	61	11	-	9	-	9	10	100 (50)
Streptococcus species	6	4	-	1	-	-	-	11 (5.5)
Klebsiella species	22	1	11	2	-	-	-	36 (18)
Escherichia coli	20	3	6	-	-	-	-	29 (14.5)
Proteus species	5	1	-	1	-	1	1	9 (4.5)
Pseudomonas species	1	-	-	1	3	1	-	6 (3)
Acinetobacter species	3	-	-	-	-	-	-	3 (1.5)
Citrobacter species	3	-	-	-	-	-	-	3 (1.5)
Enterobacter species	2	1	-	-	-	-	-	3 (1.5)
Total	123	21	17	14	3	11	11	200 (100)

Table II

Pattern of resistance of Gram positive Bacteria to Common Antibiotics

Antibiotics (Disc Concentration)	*Bacterial isolates*						*Overall Resistance*
	Staphylococcus aureus			*Streptococcus species*			
	NT	*RS*	%	*NT*	*RS*	%	%
Penicillin (1 unit)	34	33	97.1	3	2	66.7	4.6
Ampicillin (25µg)	65	47	72.3	7	5	71.4	72.2
Amoxycillin (25µg)	41	27	65.9	9	1	11.1	56.0
Co-amoxiclav (30µg)	59	22	37.3	4	0	0	34.9
Cloxacillin (5µg)	53	18	34.0	7	3	42.9	35.0
Cotrimoxazole (25µg)	54	44	81.5	9	6	66.7	79.4
Chloramphenicol (10µg)	54	10	18.5	8	2	25.0	19.7
Erythromycin (5µg)	63	17	27.0	6	0	0	24.6
Gentamicin (10µg)	69	9	13.0	5	0	0	12.2
Cefuroxime (30µg)	29	7	24.1	1	0	0	23.3
Ceftriaxone (30µg)	43	4	9.3	3	0	0	8.7
Ceftazidime (30µg)	9	2	22.2	1	0	0	20.0
Ofloxacin (5µg)	43	0	0	2	0	0	0
Ciprofloxacin (5µg)	38	0	0	2	0	0	0

NT = Number tested *RS = Resistant strain*

difference in the resistance rates between gentamicin and ceftriaxone ($X^2 = 0.41$, $df = 1$, $p = 0.521$). Klebsiella species were resistant to multiple antibiotics including ampicillin, amoxicillin, co-amoxiclav, cotrimoxazole and even to gentamicin. Eight (22.2 percent) of Klebsiella species isolated were resistant to all the antibiotics tested except to ofloxacin and ciprofloxacin. Although resistance rate was lower for other gram-negative bacteria, similar trend in resistance was observed. A strain of *Escherichia coli* (3.4 percent) and one of Proteus species (11.1 percent) were resistant to all antibiotics with the exception of the quinolones.

The antibiotic resistant pattern of isolated organisms in the present study was compared with those of previous studies in Table IV, which shows an increasing trend in antibiotic resistance.

Table III

Pattern of resistance of Gram-negative bacteria to Common Antibiotics

Antibiotics (Disc Concentration)	Bacterial Isolates												Overall Resistance
	Klebsiella spp			E. coli			Proteus spp			Pseudomonas spp			
	NT	RS	%	NT	RS	%	NT	RS	%	NT	RS	%	(%)
Amp (25ig)	15	15	100	13	12	92.3	3	2	66.7	5	4	80	91.7
Amx (25ig)	11	9	81.8	7	4	57.1	3	2	66.7	3	2	66.7	70.8
Coa (30ig)	17	13	76.5	10	2	20	3	2	66.7	3	2	66.7	57.6
Cot (25ig)	30	18	60	14	14	100	4	1	25	3	2	66.7	68.6
Chl (10ig)	7	4	57.1	2	1	50	0	0	0	4	1	25	46.2
Ery (5ig)	3	2	66.7	5	3	60	0	0	0	3	1	33.3	54.5
Gen (10ig)	20	14	70	16	1	6.3	3	3	100	3	1	33.3	45.2
Cef (30ig)	12	5	41.7	8	2	25	2	1	50	1	1	100	39.1
Cro (30ig)	15	4	26.7	10	4	40	4	1	25	5	3	60	35.3
Cft (30ig)	5	1	20	5	0	0	3	0	0	2	0	0	6.7
Ofl (5ig)	12	0	0	7	0	0	3	0	0	6	0	0	0
Cpr (5ig)	8	0	0	5	0	0	2	0	0	4	0	0	0

Amp = Ampicillin Amx = Amoxycillin Coa = Co-amoxiclav Cot = Cotrimoxazole Chl = Chloramphenicol Ery = Erythromycin Gen = Gentamicin Cef = Cefuroxime Cro = Ceftriaxone Cft = Ceftazidime Ofl = Ofloxacin Cpr = Ciprofloxacin

NT = Number tested RS = Resistant strain

Table IV

Comparison of Antibiotic resistance Patterns in Previous Studies and Present Study

Agents	*Antibiotics*	*Authors and Dates*		
		Antiabonomo et al[4] 1984-1985	*Ogala et al[9] 1996*	*Present Study 2004-2008*
		(%)	(%)	(%)
Gram-positive				
	Penicillin	75.9	NA	94.6
	Ampicillin	58.6	60	72.2
	Cloxacillin	17.2	57	35
	Cotrimoxazole	82.8	NA	79.4
	Chloramphenicol	52.2	17	19.7
	Erythromycin	33.1	NA	24.6
	Cefuroxime	NA	0	23.3
	Ceftriaxone	NA	0	8.5
Gram-negative				
	Ampicillin	80.8	90	91.7
	Cotrimoxazole	53.8	50	68.6
	Chloramphenicol	53.8	NA	46.2
	Erythromycin	DNC	NA	62.5
	Gentamicin	30.8	25	45.2
	Ceftriaxone	NA	0	35.3
	Ceftazidime	NA	0	6.7

NA = Not available *DNC – Data not clear*

Discussion

The result of this study shows that there was an increasing incidence of bacterial resistance to commonly used antibiotics with the emergence of multidrug resistant strains. Similar observations were made by workers from the western part of Nigeria,[5,10] Africa and other parts of the world.[2,11,13] Although only isolates from patients in the newborn care unit of the hospital were surveyed, the result may closely reflect the actual situation in most hospitals in the region, hence the need for widespread study to document the magnitude of the problem in the region. The penicillin group of antibiotics appeared to be losing its therapeutic relevance in the hospital, having recorded the highest resistant rate so far. The resistance rate of 72.2 percent to 91.7 percent against ampicillin clearly shows its limited usefulness in treating bacterial infections in newborns. Although resistance to cloxacillin was relatively lower, the level of resistance recorded against it is nevertheless worrisome as this drug is heavily relied upon in treating staphylococcal infections in the locality.

Erythromycin appeared to have withstood the mechanism of resistance among the isolates as only 24.6 percent of gram-positive isolates were resistant to the drug. Thus erythromycin may have a useful role in controlling gram-positive neonatal bacterial infections especially as the antibiotic has good tissue penetrability,[15] although its bacteriostatic nature and lack of readily available parenteral forms may limit its use in deep-seated, severe neonatal bacterial

infections. In addition, erythromycin is poorly concentrated in the cerebrospinal fluid[12] making it unfit for the treatment of neonatal meningitis. The cephalosporins had similarly encountered bacterial resistance and the rate of resistance development has worrying therapeutic implications especially when one considers that this group of antibiotics is generally used only as a second line therapy in our unit. A more stringent control on the use of cephalosporins may be necessary to curtail the rising resistance rates. Although as high as 23.1 percent of gram-positive isolates were resistant to cefuroxime, this drug appear to be the best in the empirical treatment of deep-seated neonatal gram-positive bacterial infections.

Recent reports[13-15] from other parts of the world have been advocating the use of quinolones in children and newborns. The zero resistance rates in this study give an overwhelming support to this idea although the issues regarding the safety of this group of drugs in the newborn are still subject to controversy. Thus, there is an urgent need for further studies on the safety profile of quinolones in newborns and children. A drug of note in this study is gentamicin which, in spite of heavy usage, still maintains a relatively low resistance rate. Hence, the continued role of gentamicin in the management of neonatal infections appears justified. However the 45.2 percent resistance rate among the gram-negative bacteria suggests the need to have other aminoglycosides as alternatives.

The emergence of multidrug resistant strains of pathogenic bacteria in this unit indicates a precarious state in the future as most of the second line drugs are not readily available in this locality. In addition, the cost of these drugs is far out of the reach of the majority of the caregivers. Thus there is an urgent need to halt the growing threat of multidrug resistant pathogens in this region by creating awareness of the existence of the condition in the community and discouraging the tendency towards self medication and poly-pharmacy. The reasons for the growing antibiotic resistance in this locality are not certain but may not be unrelated to imprudent and widespread use of antibiotics.[5,16,17] Imprudent use of antibiotics has been observed in 40 - 90 percent of prescriptions across the continents of the world.[17] In addition, antibiotics are commonly available from unauthorized providers who often reach out to people with limited access to orthodox health care.[18] These unauthorized drug providers are not trained to diagnose infections and are more likely to prescribe antibiotics at sub-therapeutic doses,[18] hence contributing significantly to the emergence of resistant strains. Furthermore, poor quality drug provides sub-inhibitory serum concentration and thus contributes to the development of resistance. Sub-standard drugs containing between 0 and 80 percent of stated label claim are not uncommon in Africa.[18] Therefore, the need to identify the sources of these sub-standard drugs, whose inflow must be stopped, is of utmost importance.

Although antibiotics are pivotal in controlling neonatal bacterial infections, prudent use of drugs and strict infection control measures are urgently needed to halt the growing threat of antibiotic resistant pathogens, while the use of non-drug therapy such as bacteriophage-derived therapy, probiotics and vaccines should be intensively explored for use in newborns so as to limit the use of antibiotics and hence, halt the growing antibiotic resistance.

Based on the information obtained from this study, some guidelines regarding therapeutic choices have emerged. Erythromycin may be employed in the empirical treatment of neonates with superficial bacterial infections. However, regarding neonates with deep-seated infections, a combination of cloxacillin plus gentamicin will continue to suffice if the infection is mild-to-moderate, while cefuroxime should replace cloxacillin in severe deep-seated infections. However, in view of the poor CSF penetrability of cefuroxime, ceftriaxone is preferable when treating neonates with meningitis. Further studies are needed to identify the most appropriate antibiotics for treating multidrug resistant pathogens in our environment.

References

1. Fleming A. Penicillins. Available on the internet at http://www.wikipedia.org.
2. Musoke RN, Revathi G. Emergence of multidrug-resistant gram-negative organisms in a neonatal unit and the therapeutic implications. *J Trop Pediatr* 2000; 46:86-91.
3. Ariffin H, Navaratnam P, Kee TK, Balan G. Antibiotic resistance patterns in nosocomial gram-negative bacterial infections in units with heavy antibiotic usage. *J Trop Pediatr* 2004; 50:26-31.
4. Ogala WN, Warrumunda RD, Zoaka HA. Analysis and outcome of admission to the special care baby unit of Ahmadu Bello University Teaching Hospital, Zaria. *Nig J Med* 1996; 5:70-3.
5. National Committee for Clinical Laboratory Standards. Antimicrobial susceptibility testing. Performance standards for antimicrobial disc susceptibility tests. NCCLS Document M@-A5, Villanova, Pa. 1993.

6. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotics susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 1966; 45: 493-6.
7. Amiebenomo CS, Yakubu AM, Bello CSS, Ewa B. Neonatal septicaemia in Zaria. *Nig Med J* 1988; 18:349-51.
8. Ogala WN, Yakubu AM, Bamgbola PO, Zoaka HA, Ewa BO. Antibiotic sensitivity in neonatal septicaemia. *Nig J Paediatr* 1996; 23:81.
9. Egri-Okwaji MTC, Iroha EO, Kesah CN, Odugbemi T. Bacterial pathogens causing neonatal sepsis inan out-born neonatal unit, Lagos, Nigeria. *Quart J Hosp Med* 1996; 6:149-52.
10. Ogunsola FT, Kesah CN, Odugbemi T. Antimicrobial resistance in Nigeria: An overview. *Nig Quart J Hosp* 1997; 57-61.
11. Vergnano S, Sharland M, Kazembe, P, Mvansambo CM, Health PT. Neonatal sepsis: an international perspective. *Arch Dis Child Fetal Neonatal Ed* 2005; 90: F220-4.
12. Hyde TB, Hilger TM, Reingold A, *et al*. Trends in incidence and antimicrobial resistance of early-onset sepsis: population-based surveillance in San Francisco and Atlanta. *Pediatrics* 2002; 110:690-5.
13. Chambers HF. Antimicrobial Agents: General Consideration. In: Goodmen & Gilman's. The Pharmacological Basis of Therapeutics. New York: McGraw-Hill 2000; 1143-255.
14. Hampel B, Hullmann R, Schmid H. Ciprofloxacin in pediatrics: worldwide clinical experience based on compassionate use - safety report. *Pediatr Inf Dis J* 1997; 16: 127-9.
15. Belet N, Haciomeroglu P, Kucukoduk S. Ciprofloxacin treatment in newborns with multi-drug-resistant nosocomial Pseudomonas infections. *Biol Neonate* 2004; 85:263-8.
16. Oyewole AIM, Akinwolere OAO, Akinkugbe FM. Use and misuse of drugs in Nigerian infants. *Nig Med J* 1987; 17: 21-9.
17. Struelens MJ. The epidemiology of antimicrobial resistance in hospital acquired infections: problems and possible solutions. *Br Med J* 1998; 317: 652-4.
18. Okeke LN. Antibiotic resistance in Africa - discerning the enemy and plotting a defense. *Afr Health* 2003; 25:10-15.