Afr. J. Med. med. Sci. (1997) 26, 51-53

Outbreak of neonatal *klebsiella* septicaemia: a review of antimicrobial sensitivities

†Juliana A. Akindele and *Ignatius O. Rotilu

†*Department of Paediatrics,* **Department of Medical Microbiology, College of Medicine, University of Ibadan, Ibadan, Nigeria*

Summary

A 10-week prospective study was undertaken to document the antibiotic susceptibilities of *klebsiella* organisms which were responsible for an outbreak of septicaemia on the neonatal units of the University College Hospital, Ibadan, Nigeria. The thirty-nine isolates obtained comprised *K. pneumoniae*, 18 (46.2%), *K. aerogenes*, 17 (43.6%), *K. edwardsii*, 3 (7.7%), and *K. oxytoca*, 1(2.5%). All the strains were sensitive to ciprofloxacin and ofloxacin, but resistant to ampicillin. The percentage of qualitative sensitivities of the *klebsiella* species to other available drugs were 41% for ceftazidime, 36% for cefotaxime, 31% for ceftriazone, 23% for cefuroxime, 21% for gentamycin, and 15% for kanamycin. Quantitative sensitivities of the three most commonly isolated sub-types to netilmycin were 63%, 36%, and 33%, respectively. A comparison with a previous antibiotic susceptibility study still showed persistent resistance to the available aminoglycosides.

Résumé

Une étudé retrospective de 10 semaines a été faite afin de documenter la susceptibilité des *klebsialla* aux antibiotoques. Ces organismes ont été responsable de plusieurs cas de scepticemie dans les unitées neonatales du Centre Hospital Universitaire de l'Université d'Ibadan, Nigeria. Trente neufs spécimens prelevés contenaient 18(46.2%) *k. pneumoniae* 17(43.6%) *k. aerogènes*, 3(7.7%) *k. edwardsii*, et 1(2.5%) de *k. oxytoca*. Toutes les souches ont été sensitive à l'action de la ciprofloxacin et de l'ofloxacin, mais résistant à l'ampicilline. Le pourcentage des sensitivitées qualitative des especes *klebsiella* aux autres médicaments disponible ont été de; 41% pour la ceftazidime, 36% pour la cefotaxime, 31% pour la ceftriazone, 23% pour la cefuroxime, 21% pour la gentamycine, et 15% pour la kanamycine. Les sensitivitiées quantitative des trois des sous-types les plus communement isolés la netilmycine ont été de 63%, 36% et 33%, respectivement. La comparaison des resultats obtenuent a celle d'une étude anterioure faite avec d'autres antibiotiques a montré une résistance persistance aux aminoglycosides disponible.

Introduction

The contribution of neonatal septicaemia to the adverse outcome of neonates, especially those of low birth weight, has been reported in the developing world [1-2]. It seems that, apart from the increased prevalence of the predisposing factors to septicaemia, another important contributor to this adverse outcome is the scarcity of potent antibiotics that are active against the prevalent causative organisms. Currently, the causative organisms reported from the tropics are mainly *enterococcal organisms* and *staphylococcus aureus*, while the rarity of group B beta haemolytic streptococcus has been previously emphasized [3-4].

This report concerns a study that was designed to document the antibiotic susceptibilities of *klebsiella* species, which were responsible for an outbreak of septicaemia in admitted neonates at the University College Hospital, Ibadan, Nigeria.

Correspondence: Dr. J.A. Akindele, Department of Paediatrics, College of Medicine, University of Ibadan, Nigeria.

Materials methods

A 10-week prospective study was carried out among neonates (inborn and outborn), aged 0-4 weeks who were admitted into the Special Care Baby Unit (SCBU), Otunba Tunwase Children's Emergency Ward and C1 2nd ward of the Paediatrics Department, University College Hospital, Ibadan, Nigeria. All babies who had risk factors for the development of septicaemia and those who had clinical features suggestive of neonatal septicaemia were included in the study. The risk factors and clinical features suggestive of neonatal septicaemia were those established by previous workers [3-7]. Cases of secondary septicaemia, i.e., following invasive investigative procedures of surgery, were excluded from the study.

Blood, aseptically obtained from the neonates, was cultured by standard methods [8-10]. The organisms obtained were subjected to drug susceptibility tests by both qualitative [11] and quantitative methods [12-13].

Qualitative method

The disc diffusion method of Bauer *et al.* [11] was used for antibiotic susceptibility testing. The discs used for this testing were impregnated with the following antibiotics: ceftazidime 30 μg, cefuroxime 30 μg, ceftriaxone 30 μg, cefotaxime 30 μg, gentamycin 30 μg, kanamycin 30 μg, ampicillin 25 μg, ciprofloxacin 55 μg, and ofloxacin 5 μg. The degree of susceptibility was noted for each antibiotic.

Quantitative method

The minimum inhibitory concentration (MIC) was determined by inoculating 0.2 ml of the overnight broth culture of the isolates, diluted 1/100 into the following gradient concentrations of antibiotics: 50, 25, 20, 15, 10, 7.5, 5, 3.75, 2.5, 1.25, and 0.25 microgr/ml as per Washington [12] and Witesbsky *et al.* [13]. Inhibition of bacterial growth at an antibiotic concentration of 25 microgram/ml or less meant that the organism was sensitive to it, whilst inhibition above this concentration denoted resistance of the organisms. The antibiotics used included ampicillin, netilmycin, ceftazidime, cefuroxime, cefotaxime, kanamycin, gentamycin, ciprofloxacin, and ofloxacin. The diluent for this purpose was Brain Heart Infusion broth (Oxoid CM 735) containing bromothymol blue as an indicator; the latter changes colour if the organism grows in the medium [12-13]. The concentration of the lowest tube that failed to show the growth of the organism gave the MIC.

The Student's *t* test was used to compare the percentage susceptibilities of a previous study with the present one. Statistical differences at 5% level ($P < 0.051$) were taken to be significant.

Result

Thirty-nine isolates were obtained from the blood of thirty-eight neonates; There were all klebsiella species and the cultures comprised *klebsiella pneumoniae* in 18 (46.2%), *klebsiella aerogenes* in 17 (43.6%), *klebsiella edwardsii* in 3 (7.7%), and *klesbiellla oxytoca* in the one remaining (2.6%) culture.

Table 1 shows the qualitative sensitivity of the bacterial isolates to the available antibiotic discs. All the isolated organisms were sensitive to ciprofloxacin and ofloxacin.

Sixteen (41%), 14 (36%), 12 (31%), 9 (23%), 8 (21%), and 6 (15%) of the klebsiella organisms were sensitive to ceftazidime, cefotaxine, ceftriazone, cefuroxime, gentamycin, and kanamycin, respectively; none of the klebsiella was sensitive to ampicillin. Quantitative drug susceptibility of all the isolates to ciprofloxacin and ofloxacin (Table 2), whilst none of the isolates was susceptible to ampicillin. *Klebsiella edwardsii* and *oxytoca* were not susceptible to ceftazidime, cefotaxine, cefuroxime, gentamycin, and kanamycin. All the subtypes of klebsiella were susceptible to netilycin as shown in Table 2.

Table 1: Qualitative antibiotic sensitivity of klebsiella isolates

Antibiotics	Klebsiella Pneumoniae	Aerogenes	Oxytoca	Edwarsii	Total
Ciprofloxacin (CIP)	18(100)	17(100)	1(100)	3(100)	39(100)
Ofloxacin (OFX)	18(100)	17(100)	1(100)	3(100)	39(100)
Ceftazidime (CAZ)	8(44)	8(47)	0(0)	0(0)	16(41)
Cefotaxine (CTX)	8(44)	6(35)	0(0)	0(0)	14(36)
Ceftnazone (CRO)	6(33)	6(35)	0(0)	0(0)	12(31)
Cefuroxime (CXM)	7(39)	2(12)	0(0)	0(0)	9(23)
Gentamycin (GN)	5(28)	3(18)	0(0)	0(0)	8(21)
Kanamycin (K)	3(17)	3(18)	0(0)	0(0)	6(15)
Ampicillin (AMP)	0(0)	0(0)	0(0)	0(0)	0(0)

Figures in parentheses are %

The susceptibility of *k. pneumoniae* and *k. aerogenes* to the remaining antibiotics were as follows: 63% and 46% to ceftazidime, 88% and 27% to cefuroxime, 63% and 18% to cefotaxine, 50% and 46% to gentamycin, and 50% and 27% to kanamycin, respectively.

Table 2: Quantitative antibiotic susceptibility (minimal inhibitory) concentration of isolates of neonatal septicaemia

Strains of klebsiella tested	Concentration of drug u/ml 50	25	20	15	10	10	Susceptibility of drug (%)	
Pneumoniae	3	0	0	0	0	5	63	
Aerogenes	6	2	2	1	0	0	46	
Edwardsii	3	0	0	0	0	0	0	Ceftazidime
Oxytoca	0	0	0	0	0	0	0	
Pneumoniae	1	2	3	1	0	1	88	
Aerogenes	8	0	1	0	0	2	27	Cefuroxime
Edwardsii	3	0	0	0	0	0	0	
Oxytoca	1	0	0	0	0	0	0	
Pneumoniae	3	0	2	0	0	3	63	
Aerogenes	9	0	0	0	0	2	18	Cefotaxine
Edwardsii	3	0	0	0	0	0	0	
Oxytoca	1	0	0	0	0	0	0	
Pneumoniae	3	1	2	2	0	0	63	
Aerogenes	7	0	3	1	0	0	36	Netilmycin
Edwardsii	2	1	0	0	0	0	33	
Oxytoca	0	1	0	0	0	0	100	
Pneumoniae	4	0	2	0	0	2	50	
Aerogenes	6	5	0	0	0	0	46	
Edwardsii	3	0	0	0	0	0	0	Gentamycin
Oxytoca	1	0	0	0	0	0	0	
Pneumoniae	4	0	1	0	2	1	50	
Aerogenes	8	2	1	0	0	0	27	Kanamycin
Edwardsii	3	0	0	0	0	0	0	
Oxytoca	1	0	0	0	0	0	0	

The overall susceptibility of all the klebsiella species isolated in this study was 35% to cefotaxime, 23% to cefuxime, 20% to gentamycin, 15% to kanamycin, and 0% to ampicillin. Corresponding figures from a previous study in the same hospital were 20%, 34%, 31%, and 3%, respectively [14]; a comparison of the two sets of figures showed no statistical difference (Table 3).

Table 3: Drug susceptibility of klebsiella: Comparison with previous study [13]

Antibiotic	SUSCEPTIBILITY % 1984[13] (n = 65)	Present (n = 39)	P	
Cefuroxime	34	23	0.24	*NS
Gentamycin	31	20	0.194	*NS
Cefotaxine	20	15	0.88	*NS
Kanamycin	13	15	0.076	*NS
Ampicillin	3	0	0.16	*NS

*NS = Not significant.

Discussion

The multiple antibiotic resistance of some strains of klebsiella organism in neonatal septicaemia has been the subject of various studies [15-17]. A previous study in our unit showed that klebsiella species constituted the single most important causatitive organism of neonatal septicaemia, accounting for 82% of the cases [3]. The present study confirmed a continued high level of resistance of klebsiella species to the available antibiotics such as ampicillin, gentamycin, and some cephalosporins. Although no statistically significant difference was noted between the qualitative antibiotic susceptibilities of the klebsiella species during this study period and those obtained from the same unit in 1981-1984, our clinical observation suggests an increasing degree of resistance. It would, therefore, appear that the commonly used aminoglycosides in the tropics, namely gentamycin and kanamycin may not be useful against this virulent organism. *Klebsiella pneumoniea* and *klebsiella aerogenes* exhibited almost identical qualitative susceptibilities to most of the drugs, whilst *k. edwardsii* and *oxytoca* were resistant to all the antibiotics except to ciprofloxacin, ofloxacin, and netilmycin. From the present study, the "safe" antibiotics among those tested to which the isolates were found to be reasonably sensitive were ceftazidime, cefuroxime, and netilmycin. This latter antibiotic, one of the newer aminoglycosides, is not available for use in Nigeria while amikacin, the other newer aminoglycoside, could not be obtained for testing in this study. Ciprofloxacin and ofloxacin, the other potentially useful antibiotics, are not recommended for paediatric usage because of lack of experience in this age group and hence, the inability to predict their side effects.

In terms of treatment of neonatal septicaemia in the tropics, it appears that the most useful of the cephalosporins may be ceftazidime and cefuroxime; netilmycin (a newer aminoglycoside) may give a promising outlook. Owing to the demonstrated resistance to such drugs as gentamycin and kanamycin, the practice of using either of them as one of the first line drugs in the treatment of such neonatal septicaemia should be temporarily suspended in areas where there is demonstrable resistance to those drugs. Additionally, the usage of the cloxacillin-gentamycin combination should be suspended while the first line drug combination should ideally be cefuroxime-netilmycin; however due to non-availability of netilmycin in the country one may be constrained to use gentamycin instead of netilmycin.

The molecular basis for the multiple antibiotic resistance of klebsiella species has been shown to be meditated by readily transferable plasmids. John *et al.* [17] used the techniques of "molecular epidemiology" to unravel the "mystery" of the two nursery outbreaks of multiple resistant *k. pneumoniae* that they had. By comparing their molecular characteristics, they discovered that the source of entry into the paediatric ward of an R plasmid containing *k. pneumoniae* (which had been endemic on the adult ward) was from the mother of a colonized infant who had *E. coil* bearing the same R plasmid as *K. pneumoniae*. The practical implications of this are that people coming to the neonatal unit need to be effectively screened and there is a need for phage-typing of the organism so as to be able to identify the source and effect the subsequent prevention of their spread. Indiscriminate use of antibiotics has

also contributed to the emergence of drug resistant organisms, a situation which is highly prevalent in this part of the world where drugs may be purchased across the counter without any prescription.

This study has identified an urgent need for similar studies using a wider range of antibiotics in other neonatal units in tropical countries where septicaemia remains a scourge and a killer of neonates. In the meantime, preventive measures must be intensified: such measures would include the provision of portable water, the avoidance of overcrowding, and an increased allocation of nursing staff to the Special Care Baby Unit so that the baby to nurse ratio will be 2:1. This is not feasible at present in a developing country such as Nigeria, as the shortage of expert nursing staff means that several ill infants are cared for by one nurse. Other measures that should be greatly emphasized and rigidly practised in order to eliminate the risk of cross infection between babies would include the regular decontamination of incubators, humidifiers, and suction apparatus; scrupulous washing of hands after handling each patient and/or his excreta; and having individualized items such as toiletries, thermometers, and disposable paper tape measures.

Lastly, the changing microbial nature of the unit should be followed by regular surveillance of cultures from babies as well as incubators and other equipment used in the nursery. This procedure is necessary for the proper antimicrobial treatment of sick newborn babies.

Acknowledgments

This project was funded by a University of Ibadan Senate Research Grant. We are grateful to Kirby-Warrick Pharmaceuticals Ltd., Mildenhell, Bunny street, Edwards, Suffolk, England, for the supply of the netilmycin used in the study. The secretarial assistance of Mrs T.O. Adegoke is appreciated.

References

1. Oruamabo RS, John CT. Antenatal care and fetal outcome especially in low birth weight in Port-Harcourt, Nigeria. Annals Trop Paediatr 1987; 3: 173-177.
2. Akintade JA, Oyedeji O, Gbadegesin RA. Trends in newborn mortality over a ten-year period at the University College Hospital, Ibadan, Nigeria. Early Child Development and Care 1992; 80: 31-41.
3. Akindele JA. Predisposing factors in neonatal septicaemia: a four year review in a Special Care Baby Unit. Nig J Pediatr 1988; 15: 33-39
4. Owa JA, Olusanya IO, Esiamai VC, Olusanya OO. Risk of neonatal septicaemia after prolonged rupture of membranes in Nigeria. Trop Geogr Med 1990; 42(3): 217-220.
5. Omene JA, Neonatal septicaemia in Benin City, Nigeria. Trop Geogr Med 1979; 31: 35-39.
6. Dawodu AH, Alausa OK. Neonatal septicaemia in the tropics. Afr J Med Sci 1980; 9: 1-6.
7. Davies PA. Bacterial infections in the feutus and newborn. Arch Dis Child 1971; 16: 1-21.
8. Grams C (1984). The differential straining of schizomycetes in tissues sections and in dried preparation. In: TD Beock, Ed. Milestone in microbiology. London: Pretentioe Hall, 1961: 215.
9. Cowan ST, Steel. In: Manual for the identification of medical bacteria: Camb University Press (Publ) 2nd edition 1974; 103.
10. Alausa KO, Montefiore D, Sogbetun AO, Ashiru JO, Onile BA, Sobayo E. Septicaemia in the tropics: A prospective epidemiological study of 104 patients with high case fatality rate. Scan J Infect Dis 1977; 9: 181.
11. Bauer AW, Kirby WMM, Sherris JC, Turch M. Antibiotic susceptibility testing by a standardised single disc method. Am J Clin Path 1966; 45: 493-497.
12. Washington JA. Antimicrobial susceptibility test. J Inf Dis 1979; 140: 216-263.
13. Witebsky FG, McLowry JD, French SS. Broth dilution minimum inhibitory concentration: Rationale for use of selected antimicrobial concentration. J Clin Micro 1979; 9: 589-595.
14. Akindele JA. Neonatal septicaemia: a four-year retrospective review in the Special Care Baby Unit at the University College Hospital, Fellowship of the West African College of Physicians (FWACP) Thesis. October 1985; 53-55.
15. Adeyemo AA, Akindele JA, Omokhodion SI. Klebsiella septicaemia, associated oesteomyelitis and septic arthritis in neonates in Ibadan, Nigeria. Annals of Trop Paediatr 1993; 13: 285-289.
16. Hable KA, Matson JM, Wheeler DJ, Hunt CE, Quie PG. Klebsiella type 33 septicaemia in infant intensive care unit. J Peadiatr 1972; 80(6): 920-924.
17. John JF, Mckee KT, Twitty JA, Scaffner W. Molecular epidemiology of sequential nursery epidemics caused by multi-resistant klebsiella pneumoniae. J Pediatr 1983; 102: 825-830.