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Extended Spectrum β Lactamase Producing *Klebsiella pneumoniae* and *Escherichia coli* in Neonatal Intensive Care Unit

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Abstract

Introduction: Neonatal Septicemia is an important cause of morbidity and mortality. As infections due to ESBL producing *K. pneumoniae* & *E. coli* are on the rise, the present study was carried out in the NICU of KIMS, Narketpally, with an aim to identify any environmental sources & the mode of transmission over a period of 3 years from August 2006 to July 2009.

Materials and Methods: A total of 264 neonates admitted with clinical features suggestive of septicemia in the NICU were studied by blood culture and CRP estimation. Antibiotic susceptibility pattern was determined. ESBL detection was done by double disc synergy test. Environmental samples from various sites (Incubators, phototherapy units, suction apparatus, trolley, door, floor, work surfaces) were collected using sterile swabs every month and processed simultaneously.

Results: Of the 264 blood cultures, 197 (75%) showed bacterial growth. *K. pneumoniae*, 64 (32.7%) was the commonest organism followed by *E. coli* 55 (28%), S. aureus 31 (16%), *Pseudomonas aeruginosa* 28 (14%), *Acinetobacter* 13 (7%), and Coagulase negative Staphylococci 6 (2.8%) respectively. *K. pneumoniae* & *E. coli* were isolated from various environmental sites at least on one occasion and consistently from phototherapy units, door & floor of the NICU. The similarity between antibiograms of ESBL producing strains of *K. pneumoniae* & *E. coli* isolates from neonates and environment of NICU were statistically significant ($P < 0.05$).

Conclusion: Wide spread use of third generation cephalosporins as a preemptive antibiotic for suspected cases of septicemia have contributed to the emergence of ESBL producing *K. pneumoniae* & *E. coli* in addition to other risk factors, both of which have extensively colonized the environment of the NICU. Repeated isolation of these two organisms from the NICU environment proves that some of the neonatal infections may be from the environment itself. Transmission can be stopped by maintaining the sterility of the NICU & hand hygiene among the mothers and health care workers.

Keywords: Neonatal septicaemia; Neonatal intensive care unit; Environment; ESBL

Introduction

Multidrug resistant Gram negative bacilli belonging to the family Enterobacteriaceae have been increasingly responsible for infections among the neonates admitted to the NICU in many countries including India. *Klebsiella pneumoniae* and *Escherichia coli* constitutes a majority of these pathogens [1,2,3]. With the emergence of ESBL producing *K. pneumoniae* and *E. coli* as the predominant pathogen, the third generation cephalosporins which have been used extensively as a life saving first line antibiotic among septicemic neonates are rendered useless, significantly increasing the morbidity and mortality in the NICUs. Many outbreaks of *K. pneumoniae* infection in the NICU have frequently been shown to have an environmental reservoir [4,5]. Irrespective of the primary source, the lower digestive tract of the colonised neonates is the main reservoir of these microorganisms and cross contamination is presumably hand carried by the attending staff [6].

In the present study, monitoring of the NICU environment in the KIMS hospital, Narketpally was performed to identify any environmental sources and to know exactly the mode of transmission of the pathogens and initiate corrective measures.

Materials and Methods

The KIMS hospital Narketpally is a tertiary care referral teaching centre. A total of 264 neonates admitted with clinical features suggestive of septicaemia in the NICU were studied by blood culture and CRP estimation. The following environmental samples were collected using sterile swabs from the NICU twice every month from August 2006 to July 2009. Swabs from the incubators, phototherapy units, suction apparatus, trolley, door, floor and work surfaces were taken twice in a month (Table 4). All the samples were transported to the microbiological lab and processed immediately and the isolates identified by standard bacteriological methods [7]. The antimicrobial susceptibility tests were done by Kirby Bauer's disc diffusion test and ESBL production was detected by double disc synergy test using commercially available ceftazidime (30 µg) and cefotaxime (30 µg) discs around an amoxy clavulanic acid (20 µg) disc (Himedia) at a radius of 30 mm. All clinical isolates of NICU patients were screened for ESBL producing *K. pneumoniae* and *E. coli*. Chi-square test was used for analysing the result.

Results

The total no. of sample obtained during the study period was 264, various microorganisms were isolated, 197 in all, of which *K. pneumoniae* accounts for 64 (32.7%) and *E. coli* for 55 (28%) (Table 1). Major-

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ity of the *K. pneumoniae* isolates (70.3%) and *E. coli* isolates (61.2%) were ESBL producers.

The *K. pneumoniae* and *E. coli* isolates were grouped based on ESBL production and antibiotic susceptibility patterns into ESBL producers and ESBL non producers (Tables 2 and 3).

Discussion

Although various risk factors for colonization of neonates with ESBL producing *K. pneumoniae* and *E. coli* in the NICU have been elucidated [1], the wide spread use of cephalosporins form the most important factor in selecting out these strains. These strains are able to survive on skin & watery surfaces and resist dessication making them easily transferable through equipments and the hands of health care workers [6,8]. The *Klebsiella* and *E. coli* species, in addition to their virulence and ability to acquire antibiotic resistance determinants [3], are able to survive on skin and watery surfaces and resist dessication [9], making them easily transferable through equipments and the hands of health care workers.

The environmental sampling revealed disturbing details of the extent to which these strains were prevalent in the NICU. Repeated isolation of bacteria also confirmed their persistent presence in the environment and that they are not occasional contaminants. Many of these strains had similar antibiogram as compared to clinical isolates suggesting common source of infection. Though molecular typing of these microorganisms can be very useful in identifying the organisms that have originated from a single strain, it was not done due to lack of facilities at our institution. The other major limitation of this study was inability to perform the Minimum Inhibitory Concentration (MIC) of the various antibiotics and cephalosporins with and without clavulanic acid for ESBL detection. MICs for the former was not performed, as the study was mainly intended to analyze the ESBLs in *K. pneumoniae* and *E. coli* and the latter due to the difficulty in procuring clavulanic acid (Table 5 and 6).

Isolate	Total No.	%	ESBL Producers No.	%
1. *Klebsiella pneumoniae*	64	32.7	45	70.3
2. *Escherichia coli*	55	28	34	61.2
3. *Staphylococcus aureus*	31	16	-	-
4. *Pseudomonas aeruginosa*	28	14	-	-
5. *Acinetobacter*	13	7	-	-
6. Coagulase negative *Staphylococci*	6	2.8	-	-

n=197

Table 1: Organism isolated from Neonates of NICU.

Isolate	Cu	Ce	Ci	G	Ak	Nt	Cf
ESBL producers	93	87	80	89	14	92	51
ESBL non producers	93	78	80	78	0	78	50

Cu: Cefuroxime; Ce: Cefotaxime; Ci: Ceftriaxone; G: Gentamicin; Ak: Amikacin; Cf: Ciprofloxacin; Nt: Netilmicin

Table 2: Antimicrobial resistance pattern of K. pneumoniae isolates from neonates (%).

Isolate	Cu	Ce	Ci	G	Ak	Nt	Cf
ESBL producers	92	92	82	90	20	90	58
ESBL non producers	91	84	77	76	0	75	45

Cu: Cefuroxime; Ce: Cefotaxime; Ci: Ceftriaxone; G: Gentamicin; Ak: Amikacin; Cf: Ciprofloxacin; Nt: Netilmicin

Table 3: Antimicrobial resistance pattern of *E. coli* isolates from neonates (%).

Site of sampling	Sample collected		ESBL producing *K. pneumoniae*			ESBL producing *E. coli*		
	No. in each survey	Total	No. of surveys positive	Total isolates	%	No. of surveys positive	Total isolates	%
Incubators	2	144	10	11	7.6	4	5	3.5
Phototherapy units	2	144	5	6	4.2	3	4	2.7
Suction apparatus	2	144	5	5	3.5	3	4	2.7
Trolley	1	72	3	4	5.6	1	2	2.8
Door	1	72	2	3	4.2	1	2	2.8
Floor	2	144	4	5	3.5	3	4	2.7
Work surfaces	2	144	5	5	3.5	2	4	2.7
			Total = 39			Total = 25		

ESBL producing *K. pneumoniae* and *E. coli* were frequently isolated from all the sites of samples from the NICU.

Table 4 details the no. of isolates from each site. It is significant to note that ESBL producing strains were always isolated from one of the incubators, phototherapy units, suction apparatus and trolley.

Table 4: Details of the Environmental sampling of the NICU.

ESBL *K. pneumoniae*	Similar antibiogram		Dissimilar antibiogram		Total No. of isolates
	No.	%	No.	%	
Neonatal isolates	22	48.9	23	51.1	45
Environmental isolates	30	76.9	9	23.1	39

Majority of ESBL producing K. pneumoniae from environment of NICU (76.9%) had similar antibiograms as that of neonatal isolates (Table 5).

The similarity between antibiogram of ESBL producing *K. pneumoniae* from neonates and environment of NICU was statistically significant (P value= 0.0158).

Table 5: Classification of ESBL producing *K. pneumoniae* from neonates and environmental samples and their antibiogram pattern.

For many years now, third generation cephalosporins, especially cephatoxime along with aminoglycosides are used in the NICU as the pre- emptive antimicrobial therapy for clinically suspected cases of septicaemia. The high prevalence of ESBL producing strains in the NICU could be related to this antibiotic policy.

The interaction between mothers admitted in the maternity wards and their neonates in the NICU, especially for breastfeeding the less unwell babies leads to the possibility of introduction and re-introduction of these bacteria from other areas in the hospital and / or colonization of these infants in the maternity wards and subsequent contamination of the NICU which needs to be evaluated. The good hand hygiene practices among these mothers before handling their babies needs to be monitored. As the NICU caters to both inborn and outborn neonates, there may be a constant replenishment of the NICU environment with these strains from other hospitals through colonised neonates, as has been demonstrated by other studies.

It is likely that the interaction of many factors, like the host, therapeutic, microbial, and environmental all contribute to the colonisation

ESBL *E. coli*	Similar antibiogram		Dissimilar antibiogram		Total No. of isolates
	No.	%	No.	%	
Neonatal isolates	15	44.1	19	55.9	34
Environmental isolates	20	80	5	20	25

Majority of ESBL producing *E. coli* from environment of NICU (80%) had similar antibiograms as that of neonatal isolates (Table 6).
The similarity between antibiogram of ESBL producing *E. coli* from neonates and environment of NICU was statistically significant (P value= 0.0123).

Table 6: Classification of ESBL producing *E. coli* from neonates and environmental samples and their antibiogram pattern.

of sick babies [10]. So it is important to take steps to reduce the multi-drug resistant pathogens like ESBL producing *K. pneumoniae* and *E. coli* in the environment of the NICU. One of the important aspects of these measures is to restrict the widespread use of broad spectrum cephalosporins for empiric therapy.

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