International Journal of Advances in Medicine
Sarangi KK et al. Int J Adv Med. 2017 Jun;4(3):678-681
http://www.ijmedicine.com
pISSN 2349-3925 | eISSN 2349-3933
DOI: http://dx.doi.org/10.18203/2349-3933.ijam20171995

Original Research Article

Drug resistance pattern analysis of various organisms isolated from neonatal intensive care unit of a tertiary care hospital in Odisha, India

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Received: 12 April 2017
Revised: 23 April 2017
Accepted: 24 April 2017

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ABSTRACT

Background: Neonatal septicaemia is characterized by systemic signs and symptoms of generalized bacteraemia with a positive blood culture in the first four weeks of life. This study was designed to detect Multi Drug Resistant pattern from organisms in neonatal septicaemic cases. The aim of the study is to find the antibiotic sensitivity pattern and drug resistance pattern (ESBL, MBL, Carbapenemase, AmpC β-lactamase, MRSA) among the organisms isolated.
Methods: This prospective study was carried out in the Department of Microbiology in association with Dept. of Paediatrics and NICU, of Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, India during the period from November 2012 - April 2014. The study was conducted on 250 patients. The inclusion criteria are neonates with suspected septicaemia, admitted to NICU, of KIMS, Bhubaneswar, Odisha, India. Two ml of venous blood from each neonate was collected & cultured by automated method. Phenotypic confirmatory tests for ESBL production and MBL detection was done as per CLSI guideline. MRSA detection was done by Cefoxitin disk (30 mcg) screen test.
Results: All gram positive pathogenic isolates were sensitive to Linezolid, Tigecycline and Vancomycin. Maximum resistance was seen against Benzylpenicilin and Ampicillin. Among the gram-negative isolates maximum antibiotic sensitivity was observed for Tigecycline, Levofloxacin and amikacin & resistance was maximum for cefadroxil and Ampicilin. Among the gram negative bacterial pathogens ESBL production was maximum by *E.coli* (75%) and *Burkholderia cepacia* (75%) followed by Enterobacter cloacae (66.7%) and *Acinetobacter iwoffi* (50%). Metallo-beta- lactamase production was seen maximum in *Acinetobacter iwoffi* (100%) followed by *Burkholderia cepacia* (75%). Methicillin resistance was seen maximum by *S. epidermidis* (58.3%) followed by *S. haemolyticus*, *S. aereus*, *S. warneri* and *S. hominis.*
Conclusions: This study facilitated the screening of MRSA, ESBL and MBL producing pathogens which are an emerging problem. Long term surveillance is needed to combat this emerging global challenge.

Keywords: Drug resistance, ESBL, MBL, MRSA, Neonatal sepsis

INTRODUCTION

Early and appropriate antimicrobial therapy is one key determinant in the ultimate outcome of the patient with sepsis. This may help in reducing mortality and morbidity. Due to changing pattern of antibiotic use and changes in life style, the spectrums of organism that causes neonatal sepsis changes over times and varies from region to region. Epidemiological data indicates differences in incidence, risk factors, antimicrobial susceptibility pattern in cases of neonatal septicaemia in developed and developing countries. Routine

antimicrobial prophylaxis may lead to emergence of multidrug resistant organisms and fungal infections which are difficult to treat. Recognition of clinical features associated with neonatal septicaemia would help in starting empirical therapy. However specific therapy based on the antibiogram of the isolate and antibiotic policy depending on the resistance pattern will definitely improve the therapeutic outcome.[1] The primary objective of the study is to find the antibiotic sensitivity pattern of pathogens isolated from neonatal septicaemic cases and to find the various drug resistance patterns (ESBL, MBL, MRSA) observed among the multidrug resistant pathogens.

METHODS

This prospective study was carried out in the Department of Microbiology in association with Dept. of Paediatrics and NICU, of Kalinga Institute of Medical Sciences, Bhubaneswar Odisha, India during the period from Nov. 2012 - April 2014 where drug resistance pattern are seen after the isolates obtained from automated blood culture by BacT/Alert and VITEK2 method. The study was conducted on 250 patients.

Inclusion criteria

Neonates with suspected neonatal sepsis admitted to Dept. of Paediatrics and NICU, of Kalinga Institute of Medical Sciences (KIMS), Bhubaneswar, India.

Detection of E.S.B.L

Phenotypic confirmatory tests for ESBL production was done as per CLSI guideline where cefotaxime (30 µg) and ceftazidime disks (30 µg) with and without clavulanic acid (10 µg) were put, at a distance of 20mm apart on Muller-Hinton agar after lawn culture with a suspension of 0.5 McFarland standard test organism and incubated overnight for phenotypic confirmation of the presence of ESBL. A difference of ≥5 mm between the zone diameters of either of the cephalosporin disks and their respective cephalosporin/ clavulanic acid disk was taken to be the phenotypic confirmation of ESBL production.[2] ESBL producing *Klebsiella pneumoniae* ATCC 700603 was taken as positive control and *Escherichia coli* ATCC 25922 was used as negative control.

Detection of Metallo β- lactamase (MBL)

This was done also by EDTA combination disc test and was done by a lawn culture of test organism which was made on Muller-Hinton agar plate using a 4-6-hour broth culture adjusted to 0.5 McFarland's turbidity. Two imipenem and 2 meropenem discs were placed on inoculated plates. 5µL of 0.5M EDTA solution (equal to 930µgm of EDTA) was added to one imipenem disc, and another 5µL of 0.5M EDTA solution was added to one Meropenem disc. In addition, a plain filter paper disc containing 5µL of 0.5M EDTA was placed on the agar plate to rule out EDTA inhibition.

After overnight incubation at 370°C, the diameter of zone of inhibition around carbapenem discs alone and those with 0.5M EDTA was recorded and compared. An increase in zone diameter of ≥7mm in the presence of 930µgm of EDTA compared to the diameter with the carbapenems tested alone was considered to be positive test for MBL.[2]

Detection of MRSA

Cefoxitin disk screen test

By definition, all methicillin-resistant *S. aureus* (MRSA) isolates carry the mec A gene, which confers resistance to all beta - lactam antibiotics, including cephalosporins and carbapenems. Apart from using molecular methods to detect the mec A gene directly, the most accurate phenotypic test for the presence of the mec A gene in *S. aureus* is the cefoxitin disk diffusion test. In this study, Cefoxitin is used because it is a more potent inducer of mec A expression than other agents such as oxacillin and the test results are relatively easy to interpret. A lawn culture was done from the test isolate on Mueller Hinton agar under standardized conditions with a cefoxitin disk (30 mcg). According to the Clinical and Laboratory Standards Institute (CLSI), a zone of growth inhibition around the cefoxitin disk of ≥22 mm rules out MRSA; a zone size <22 mm indicates that the mecA gene is present and the isolate reported as MRSA.

RESULTS

Out of 250 clinically suspected septicaemic cases of neonates 82 (32.8%) are culture positive cases. Maximum resistance was seen against Benzylpenicilin (86.6%) and Ampicilin (71.2%) Among the gram negative bacterial pathogens isolated from septicaemic cases ESBL production was maximum by *E.coli* (75%) and *Burkholderia cepacia* (75%) followed by *Enterobacter cloacae* (66.7%) and *Acinetobacter iwoffi* (50%) (Table 1).

Table 1: Bacterial pathogens producing ESBL.

Name of bacteria	Number	%
Enterobacter cloacae (n=6)	4	66.7
Burkholderia cepacia (n=4)	3	75
Acinetobacter iwoffi (n=2)	1	50
S. paratyphi A (n=2)	0	0
E. coli (n=8)	6	75
Total (n= 22)	14	63.6

Metallo- beta- lactamase production was seen maximum in *Acinetobacte iwoffi* (100%) followed by *Burkholderia cepacia* (75%). Other gram negative isolates like *E.coli*, *Enterobacter cloacae* and *S. paratyphi* A did not show any MBL production (Table 2).

Table 2: Frequency of metallo Beta-lactamase producing bacterial pathogens.

		No.	%
Enterobacter cloacae	(n=6)	0	0
Burkholderia cepacia	(n=4)	3	75
Acinetobacter. lwoffi	(n=2)	2	100
S. paratyphi A	(n=2)	0	0
Esch. coli	(n=8)	0	0
Total	22	5	22.7

Methicillin resistance was seen maximum by *S. epidermidis* (58.3%) followed by *S. hemolyticus* (50%), *S. aereus* (40%), *S. warneri* (33.3%) and *S. hominis* (33.3%) (Table 3).

Table 3: Bacterial pathogens showing methicillin resistance.

Name of bacteria	Number	%
S.haemolyticus (n=28)	14	50.0
S.epidermidis (n=12)	7	58.3
S.warneri (n=3)	1	33.3
S.hominis (n=3)	1	33.3
S.aureus (n=6)	2	40.0
Total (n=52)	25	48.08

DISCUSSION

In the present study, lowest sensitivity was found to Penicillin (7.41%) and Ampicillin (18.52%) which is close to the findings of our study. In present study Resistance was maximum for cefadroxil (86.4%), Ampicillin (72.7%), cefuroxime (68.2%) and Gentamicin (54.5%) which was also nearer to the said study where maximum resistance was observed for Cefadroxil (86.4), Ampicillin (72.7%) and Cefuroxime (68.25%). Maximum resistance was found against Ampicillin (81.5%) and Gentamicin (85.2%). Among the gram negative bacterial pathogens isolated from septicaemic cases, ESBL production was maximum by *E.coli* (75%) and *Burkholderia cepacia* (75%) followed by E*nterobacter cloacae* (66.6%) and *Acinetobacter iwoffi* (50%) which is nearer to the study conducted by Amita Jain et al where ESBL production was seen in 73.45% of *Enterobacter spp* and 63.6% of *Esch.coli*.[3]

Study conducted by Subhasree Roy et al also showed 75% *Esch.coli* as ESBL producers.[4] But in another study by Sashi Gandhi et al ESBL production was seen in only 52.9% of *Esch.coli.*[5] In present study Metallo- beta-lactamase production was seen maximum by *Acinetobacter iwoffi* (100%) followed by *Burkholderia cepacia* (75%). Other gram negative isolates like *E.coli, Enterobacter cloacae* and *S. paratyphi* A did not show any MBL production. Study carried out by Clare Franklin et al also showed 100% MBL production by *Acinetobacter spp* but overall MBL production was 62.6% which is in contrast to our finding of 22.7%.[6] Methicillin resistance was seen maximum by *S. epidermidis* (58.3%) followed by *S. haemolyticus* (50%), *S. auereus* (40%), *S. warneri* (33.3%) and *S. hominis* (33.3%) and overall Methicillin resistance production was 48.8% in our study.

This finding is nearer to the study conducted by F. Motara et al where among the gram-positive isolates, Methicillin resistance was found in 58.7% cases and *S. epidermidis* in 55.5% cases.[7] Similarly in the study conducted by Shivani Saxena et al Methicillin resistant CoNS were detected in 54.5% cases amongst which *S. epidermidis* was the commonest isolate.[8] Kamble Arati et al showed only 33.3% isolates to be MRSA producers.[9]

CONCLUSION

This study facilitated the screening of MRSA, ESBL and MBL producing pathogens which is an emerging problem in our institution as far as resistance to commonly used antibiotics are concerned. Long term surveillance is needed to describe the varied pathogens causing neonatal sepsis as well as their changing antibiotic susceptibility profile.

ACKNOWLEDGEMENTS

Authors would like to thank all the staffs of Department of Microbiology and Paediatrics as well as to the management of Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, India for their constant support and encouragement in completing the project.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

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Cite this article as: Sarangi KK, Pattnaik D, Mishra SN, Nayak MK, Jena J. Drug resistance pattern analysis of various organisms isolated from neonatal intensive care unit of a tertiary care hospital in Odisha, India. Int J Adv Med 2017;4:678-81.