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Multidrug Resistance and Extended Spectrum Beta Lactamase Production in *Klebsiella* Species Isolated From Cases of Neonatal Septicaemia

Gargi Dangre-Mudey[1], Neelima S. Tankhiwale[2] and R. P. Fule[3]

Department of Microbiology, Jawaharlal Nehru Medical College, Sawangi (M), Wardha, Maharashtra, India
E-mail: [1]<gargimudey@hotmail.com>, [2]<nst_dhanu@live.com>, [3]<rameshfule2010@gmail.com>

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ABSTRACT Worldwide Neonatal intensive care units are facing increasingly rapid emergence of antibiotic resistant bacteria, commonest among them being Multidrug Resistant (MDR) and Extended Spectrum Beta Lactamase (ESBL) producing *Klebsiella species* which creates serious therapeutic problems .So we conducted this study with the objective of accessing the prevalence of MDR and ESBL production in *Klebsiella species* isolated from cases of neonatal septicaemia. 100 non-repeat clinical isolates of *Klebsiella species* isolated from 396 blood samples of suspected cases of neonatal septicaemia in NICU from December 2008 to March 2010 were studied for MDR and ESBL production by CLSI guidelines. Among 100 isolates 96 percent were MDR, 95 percent ESBL producer by screening and 75 percent ESBL producer by confirmatory test. Detection for ESBL should be carried out as a routine by PCDDT as it is simple and cost effective test.

INTRODUCTION

The neonatal intensive care units (NICUs) today face one common problem of tackling septicaemia (Shaw et al. 2007). The incidence of neonatal septicaemia is 1 to 10 per thousand live births (Stoll 2004). Neonatal septicaemia is one of the commonest causes of neonatal mortality in the developing world accounting for 30-50 percent of 5 million neonatal deaths per year (Stoll 1997).

Multidrug resistant Gram negative bacilli belonging to the family enterobacteriacae have been increasingly responsible for infections among the neonates admitted to the NICU in many countries including India and *Klebsiella pneumonia* constitutes a majority of these pathogens (Krishna et al. 2007). ESBLs are more prevalent in *Klebsiella species* than any other enterobacterial species (Jain and Mondal 2007; Hansotia et al. 1997; Manchanda et al. 2005). Extended spectrum beta lactamases are enzymes that mediate resistance to extended -spectrum (third generation) cephalosporins (for example, ceftazidime, cefotaxime, and ceftriaxone) and monobactams (for example, aztreonam) but do not affect cephamycins (for example,cefoxitin and cefotetan) or carbapenems (for example,, meropenem or imipenem) (CDC 1999).

ESBL producing *Klebsiella species* were first reported in 1983 from Germany and since then a steady increase of strains resistant to cephalosporins has been seen. The emergence of ESBL producing strains derived from mutation in TEM and SHV enzymes which are present in 75 per cent of enterobacteriacae isolates is documented (Jain and Mondal 2007).

In India high prevalence of ESBL producing *Klebsiella* strains has been reported by various groups. Reported frequency of ESBL producing *Klebsiella species* from India ranged between 13 to 87 percent (Hansotia et al. 1997; Manchanda et al. 2005; Tankhiwale et al. 2004; Shubha and Ananthan 2002; Shukla 2004; Mathur et al. 2005; CLSI 2007).

The prevalence of infections due to MDR and ESBL producing gram negative bacteria has increased in recent years. Institutional outbreaks especially in ICUs are increasing because of selective pressure due to heavy use of expanded spectrum cephalosporins and lapses in effective

Address for correspondence:
Dr. Gargi Mudey
Assistant Professor,
Department of Microbiology,
Jawaharlal Nehru Medical College,
Sawangi (M), Wardha,
Maharashtra, India
E-mail: gargimudey@hotmail.com

control measures. Surveillance of patients in ICUs for MDR and ESBL production will help in early detection and control practices.

This study was undertaken to find out the prevalence of MDR and ESBL production in *Klebsiella species* isolated from cases of neonatal septicaemia in the Acharya Vinoba Bhave Rural Hospital, Sawangi (M) Wardha and to study sensitivity and resistance pattern in *Klebsiella species* isolates.

MATERIAL AND METHODS

A prospective study of 100 non-repeat clinical isolates of Klebsiella species isolated from 396 blood samples of suspected cases of neonatal septicaemia in NICU of AVBRH, Sawangi (M) Wardha, from December 2008 to March 2010 for MDR and ESBL production was done by following the CLSI guidelines (CLSI document M 100-S17 .Vol.27 No. 1, 2007).

1. Antimicrobial Susceptibility: Susceptibility tests were performed using the Kirby-Bauer disc diffusion method as per CLSI guidelines (CLSI 2007).

Antimicrobial discs used were ampicillin (10 µg), amoxicillin-clavulanic acid (30 ug), gentamicin (30ug), amikacin (30ug), ciprofloxacin (5µg), cotrimoxazole (25µg), cefuroxime (30 µg), cefotaxime (30µg), ceftazidime (30µg), imipenem (30µg). The discs were obtained from Himedia laboratory. Quality control was achieved by using Klebsiella ATCC 70063.

2. Multidrug Resistance*:* Isolates resistant to three or more antimicrobials of different classes were considered MDR.

3. Screening for ESBL: An inhibition zone of ≤ 22mm for ceftazidime (30µg) and ≤ 27mm for cefotaxime (30µg) indicated that the isolate was a potential ESBL producer.

4. Confirmation for ESBL*:* Done by Phenotypic confirmatory disc diffusion test (PCDDT). Four discs containing cefotaxime (30µg), cefotaxime/clavulanic acid (30µg/10µg), ceftazidime (30µg) and ceftazidime/ clavulanic acid (30µg/10µg) were used. A ≥ 5mm increase in zone diameter for antimicrobial tested in combination with clavulanic acid versus its zone when tested alone confirmed ESBL production.

RESULTS

Ninety-six percent isolates were MDR. Maximum resistance was observed with ampicillin (100 percent), followed by cefuroxime (86 percent) and cotrimoxazole (80 percent). 95 percent isolates were ESBL producer by screening and 75 percent were ESBL producer by confirmatory test. All ESBL were MDR. Among ESBLs maximum resistance was observed with ampicillin (100 percent), followed by cefuroxime (90.66 percent), cotrimoxazole (86.66 percent). Among ESBL 96 percent strains were sensitive to imipenem; 33.33 percent to amikacin; 32 percent to ciprofloxacin.

Isolates negative in screening were all negative in confirmatory test. But small percentage of isolates (21.05 percent) that were positive in the screening test were negative for ESBL production when tested by confirmatory method. Among 75 isolates that were ESBL producers by confirmatory tests 2(2.66 percent) were sensitive to cefotaxime, 7(9.30 percent) were sensitive to ceftazidime and 8(10.60 percent) were sensitive to both cefotaxime and ceftazidime.

Table 1 shows the sensitivity pattern among ESBL producers and non-ESBL producers.

Table 1: Comparison of antimicrobial resistance pattern of ESBL producer (n=75) and non-ESBL producer (n=25) in *Klebsiella* sp.

Antibiotics	*Resistance among ESBL producer (n=75)*	*Resistance among non ESBL producer (n=25)*	*z value*	*Significance*
Ampicillin	100	100	NA	NA
Amoxycillin-clavulanic acid	64	69.33	1.89158	NS
Gentamicin	80	86.66	2.93660	S
Amikacin	64	66.66	0.99385	NS
Ciprofloxacin	65	68	1.08941	NS
Cotrimoxazole	80	86.66	2.93669	S
Cefuroxime	86	90.66	2.38904	S
Ceftazidime	75	80	2.00	S
Cefotaxime	85	92	3.3955	S
Imipenem	3	4	3.77964	S

S = Significant (P < 0.05); NS = Not Significant; NA=Not applicable

Among all the tested antimicrobials, the antimicrobial resistance was significantly (P < 0.05) higher in ESBL producers than in non-ESBL producers for gentamicin, cotrimoxazole, cefuroxime, ceftazidime, cefotaxime and imipenem.

DISCUSSION

Klebsiella sp. isolated from neonates with septicaemia is a successful opportunistic pathogen. Multidrug resistance and ESBL production is a very common activity in these organisms. The present study was conducted to examine the prevalence of MDR and ESBL producing *Klebsiella strains* recovered from with septicaemia in NICU of AVBRH.

In the present study MDR in *Klebsiella* species was found to be 96 percent which is in concordance with various studies from India as reported by Arora et al. (2006) in Amritsar (71 percent), Manchanda et al. (2005) in Delhi (86.6 percent) and Subha A et al. (2002) in Chennai (100 percent).

Resistance was observed to commonly used antibiotics such as ampicillin, amoxicillin-clavulanic acid, gentamicin, amikacin, ciprofloxacin, cotrimoxazole, cefuroxime, cefotaxime, ceftazidime. The presence of MDR may be related to the dissemination of antibiotic resistance among hospital isolates of *Klebsiella sp.*

Sirot et al. (1995) reported 14-16 percent prevalence of ESBL in France and England. Jacoby et al. (1996) in his study from United States reported prevalence of 5 percent. Saurina et al. (2000) found prevalence of ESBL production 44 percent in various isolates from hospital in Brooklyn. Mathai et al. (2001) in the study of various samples from 5 medical centres in US found 4.8-6 percent of enterobactericeae isolates ESBL producers. Winokur et al. (2001) found that ESBL phenotypes were more prevalent in Latin America (45.5 percent) followed by western pacific region (24.6 percent), Europe (22.6 percent), US (7.6 percent) and Canada (4.9 percent). Cordero et al. (2004) carried out study in Canada and reported the same prevalence of 4.9 percent. Prevalence in these studies is much lower than found in the present study and also other studies carried out in various parts of India.

Hansotia et al. (1997) reported ESBL production in 76.5 percent enterobactericeae isolates from various isolates in hospital at Nagpur from central India. Subha et al. (2002) in the study of various clinical isolates in Chennai from South India found ESBL production in 25.8 percent isolates. Jain et al. (2003) in the study from Lucknow, north India, reported 86.6 percent of gram negative bacteria isolated from neonatal septicaemia ESBL producers. Jain et al. (2007) in the study from the same hospital found ESBL in 58 percent *Klebsiella* sp. from neonatal septicaemia cases. Vinodkumar et al. (2004) in the study from Gulbarga reported prevalence of 13.54 percent in various clinical isolates. Shobha et al. (2009) found 41 percent of urinary isolates ESBL producers in a study at Manipal.

The researchers observed a high rate of ESBL production by *Klebsiella sp.* which may be due to the selective pressure imposed by extensive use of antimicrobials. The indiscriminate use of cephalosporins is responsible for the high rate of ESBL producing microorganisms.

Such a broad spectrum resistance is a cause of concern and necessitates the restricted use of extended spectrum cephalosporins and other antibiotics and a trial of other suitable alternative.

Isolates negative in screening were all negative in confirmatory test. But small percentage of isolates (21.05 percent) that were positive in the screening test were negative for ESBL production when tested by confirmatory method. Therefore negative results are a better guide than positive results and confirmation of all positive results by screening should be done to prevent unnecessary avoidance of conventional beta-lactams.

Carbapenems are considered the drug of choice for the treatment of infections due to ESBL producing Klebsiella isolate. In our study 3/75 isolates showed resistance to carbepenam is a serious concern. The resistance may be due to reduced level of drug accumulation or increased expression of pump efflux or may be due to the production of metallo-β-lactamase as seen in pseudomonas spp (Amita Jain and Rajesh Mondal 2007). Thus there is a need for developing novel agents in the near future otherwise these organisms may lead to therapeutic dead end.

CONCLUSION

Detection for the ESBL should be carried out as a routine by PCDDT as it is simple and cost effective test.

RECOMMENDATIONS

Infection control practices like hand washing, barrier precautions, isolation of infected

patients, empirical use of antibiotics, antibiotic cycling could help in control of the emergence and spread of ESBL producing bacteria.

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