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Comparison of Methods for Detection of Extended Spectrum Beta Lactamases Production by Escherichia coli and Klebsiella Isolates from Neonatal Sepsis Cases

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Abstract

Background: ESBL production in critical conditions especially in neonatal sepsis is a burgeoning problem and their detection poses hindrance in establishing the prompt diagnosis.

Aim: This study was carried out to unearth the effective and easy standard method to identify ESBL production in *Escherichia coli* and *Klebsiella* species isolated from neonatal sepsis cases.

Methodology: 382 neonatal sepsis cases were subjected to blood culture and the isolates were identified and screened as per CLSI guidelines 2016 while the confirmation were done by combined disc diffusion test, Minimum inhibitory concentration test, Double Disc approximation test and E-test to check their efficacy.

Results: Blood culture positivity was found to be 32.46% (EONS- 47.38% &LONS- 52.42%) out of which 58.87% were Gram negative isolates, 37.9%were Gram positive isolates and 3.22% were *Candida spp*. *E.coli* and Group B *Streptococci* were more common in EONS while *Klebsiella spp*, CoNS and *Pseudomonas aeruginosa* were more common in LONS cases. Out of 54.72% presumptive ESBL producers, phenotypic confirmation by CDDT and MIC reduction test were done in 45.28% isolates (*E. coli;* 81.25%&*Klebsiella* species; 84.6%) while 68.75% & 69.23% for *E.coli* and *Klebsiella* species respectively were confirmed by E strip test. The DDAT were positive for 62.5% & 61.5% number of cases respectively. The sensitivity, specificity, PPV and NPV were found to be 100% each for MIC, for DDAT (70.83%, 100%, 100% and 42%) and for E test to be (83.33%, 100%, 100% and 55.56%).

Conclusion: Low specificity of screening test reflects detection of many false positive strains and low sensitivity of tests signals many missed identification. This study suggested the use of E test is better method to confirm screening positive ESBL isolates along with CDDT and MIC reduction test at microbiology laboratory.

Keywords: CDDT, *E.coli*, ESBL, *Klebsiella*, neonatal Sepsis

Introduction

Neonatal sepsis remains a major contributor to neonatal deaths in the developing as well as in developed countries [1]. Sepsis due to bacterial infection during the first 28 days

of life has remained a major cause of infant morbidity and mortality despite the ever developing technological advancements in life supportive therapy and broad spectrum antimicrobial agents.

The early and prompt diagnosis of neonatal sepsis still poses great difficulties. The clinical symptomatology of neonatal septicemia is imitated by assortment of other clinical conditions affecting the neonates. Based on the timing of the onset of infection neonatal sepsis has been classified into early-onset neonatal sepsis (EONS) and late-onset neonatal sepsis (LONS). EONS is defined as the onset of sepsis till 72 hours after birth while LOS presents after 72 hours of life [2]. Recent reviews revealed that the pathogens most often responsible for neonatal sepsis in developing countries differ from those isolated in developed countries. Generally, Gram negative bacteria are more frequent and are chiefly represented by *Klebsiella spp, Escherichia coli, Pseudomonas aeruginosa*. Of the Gram positive organisms, *Staphylococcus aureus* and Coagulase negative *Staphylococci* (CoNS) are most commonly isolated organism. *Group B Streptococci, E. coli, Enterobacter, Enterococcus* and *Listeria* spp are mostly associated with EONS. *Klebsiella, Acinetobacte*r and *S. aureus* are associated with EONS and LONS both. *Pseudomonas, Salmonella,* and *Serratia* spp. are more often associated with LONS while CoNS are found in both [3].

The extensive use of third generation cephalosporins as first-line drugs in these cases adds on the burden. The multi-resistance in Gram-negative bacteria maybe associated with production of extended spectrum β-lactamase (ESBL), AmpC β-lactamases and metallo β-lactamases. ESBL producing isolates, in addition to being resistant to β-lactam antibiotics including third-generation cephalosporins and aztreonam often exhibit resistance to other classes of drugs such as aminoglycosides, cotrimoxazole, tetracycline and fluoroquinolones [4].

ESBLs can be detected by phenotypic as well as genotypic methods. Routine detection at clinical laboratory depends upon the phenotypic methods established by CLSI guidelines. The recommended strategy for ESBL detection includes initial screening followed by corroboration. Screening tests that are recommended by CLSI are disk diffusion and broth microdilution [5]. Disk diffusion utilizes cefotaxime or cefodoxime or ceftadizime or aztreonam disk. Use of more than one antimicrobial disk for screening improves the sensitivity of ESBL detection. Phenotypic confirmation can be done by Disk diffusion tests like combined disc diffusion method [5].

Confirmatory testing requires the use of either cefotaxime or ceftadizime alone and in combination with clavulanic acid. These tests are standardized for *Escherichia coli, Klebsiella pneumonia, Klebsiella oxytoca* and *Proteus mirabilis*. Other confirmation methods are combined Double disk synergy test (DDST), ESBL gradient test, E test for ESBL, Three dimensional test etc [6, 7].

The increased prevalence of *Escherichia coli and Klebsiella species* producing ESBLs in fatal conditions like neonatal sepsis creates an immense need for laboratory testing methods which will precisely identify the presence of these enzymes in clinical isolates. Hence, the current study was undertaken to detect ESBL producers by using CLSI screening test, combined disc diffusion test (CDDT), Jarlier double disc synergy (approximation) test (DDST), and Ezy MIC test (commercial test) and to compare their efficiency.

Materials& Methods

A total of 382 blood samples were collected and processed in this cross sectional study which was carried out in the Department of Microbiology of Rohilkhand Medical College and Hospital, Bareilly in alliance with Santosh

Medical College Hospital, Ghaziabad from May 2015 to May 2017 after ethical clearance by institutional ethics committee of both the institutes.

Blood samples (1-2 ml) from the neonates were collected by venipuncture under aseptic precautions and the samples were inoculated on blood culture bottle containing Brain Heart Infusion (BHI) broth (Himedia, Mumbai). The broths were incubated aerobically at 37˚ C for 7 days and subcultured onto 5% sheep blood agar and Mac Conkey agar. The culture isolates on the both the agar plates were identified by colony characteristics, Gram staining, motility and standard biochemical tests for confirmation of *Escherichia coli* and *Klebsiella species*. AST was performed by Kirby Bauer disc diffusion method as per Clinical Laboratory and Standard Institute (CLSI) [8] using Mueller Hinton Agar plates (MHA) and commercially procured antibiotic discs (Himedia).

Screening Test: According to the CLSI guidelines by "Disc Diffusion Method". The isolates showing inhibition zone size of ≤ 22 mm with Ceftazidime (30 µg), ≤ 25 mm with Ceftriaxone (30 µg), and ≤ 27 mm with Cefotaxime (30 µg) were identified as potential ESBL producers [5].

Confirmation of ESBLs

- "**Combined Disc Diffusion Method**" (CDDT): This test was done by using a disk of Ceftazidime (30µg) alone and a disk of Ceftazidime + Clavulanic acid (30 µg/10 µg). A disk of Cefotaxime (30µg) alone and a disk of Cefotaxime+ Clavulanic acid (30 µg/10 µg) were also used. Both the disks were placed at least 25 mm apart, center to center, on a lawn culture of the test isolate on Mueller Hinton Agar (MHA) plate and incubated overnight at 37°C. A difference in zone diameters with and without clavulanic acid of ≥ 5 mm confirmed ESBL production [7].
- **Determination of MIC by Agar Plate Dilution Method:** For ceftazidime a break point of MIC, ≥16 µg/ml was taken ESBL positive. MIC range was followed according to the CLSI guidelines (CLSI, 2010) [8].
- **Double Disc Approximation Test for ESBL**: Synergy was determined between a disc of amoxicillin-clavulanate (20 µg/10 µg) (augmentin) and a 30-µg disc of each third-generation cephalosporin test antibiotic placed at a distance of 20 mm from center to center on a Mueller Hinton Agar (MHA) plate swabbed with the test isolate as described by Jarlier *et al* [9].
- **E Test (Triple ESBL Detection Ezy MICTM Strip (MIX+/MIX) EM079)** (Ceftazidime, Cefotaxime & Cefepime Mix: 0.125-16) (Ceftazidime, Cefotaxime & Cefepime Mix + Clavulanic acid: 0.032- 4). **(ESBL & AmpC detection Ezy MIC™ Strip (MIX+/MIX) EM081) MIX +:** Ceftazidime, Cefotaxime, Cefepime, Cloxacillin + Clavulanic acid (0.032 - 4) MIX: Ceftazidime, Cefotaxime, Cefepime & Cloxacillin (0.125 -16) (Himedia, Mumbai) Standard bacterial suspension was made inoculated on MHA plate. Triple ESBL detection strip was placed on Muller Hinton agar plate. Plates were incubated overnight at 37°C aerobically. Presence of ESBL was confirmed by the appearance of a phantom zone or when the minimum inhibitory concentration (MIC) of antibiotic mixture side was reduced by ≥ 8 times in the presence of β-lactamases inhibitor and the AmpC is interpreted as described in kit literature.

The data were entered and analyzed using Statistical Package for Social Sciences (SPSS, version 22). Statistical analyses were performed using descriptive statistics such as frequency, percentage, mean and standard deviation. The value P ≤ 0.05 was considered to be statistically significant. Sensitivity, specificity, positive and negative

predictive value were used for validity testing of different phenotypic tests.

Results

Of the 382 blood samples from neonates processed, 124 (32.46%) samples were culture positive. Of which positivity in males were n=78/227 (34.36%) and n=46/155 (29.67%) in females as shown in Fig 1. The association between the sexes and blood culture in the study was not significant (p>0.05).

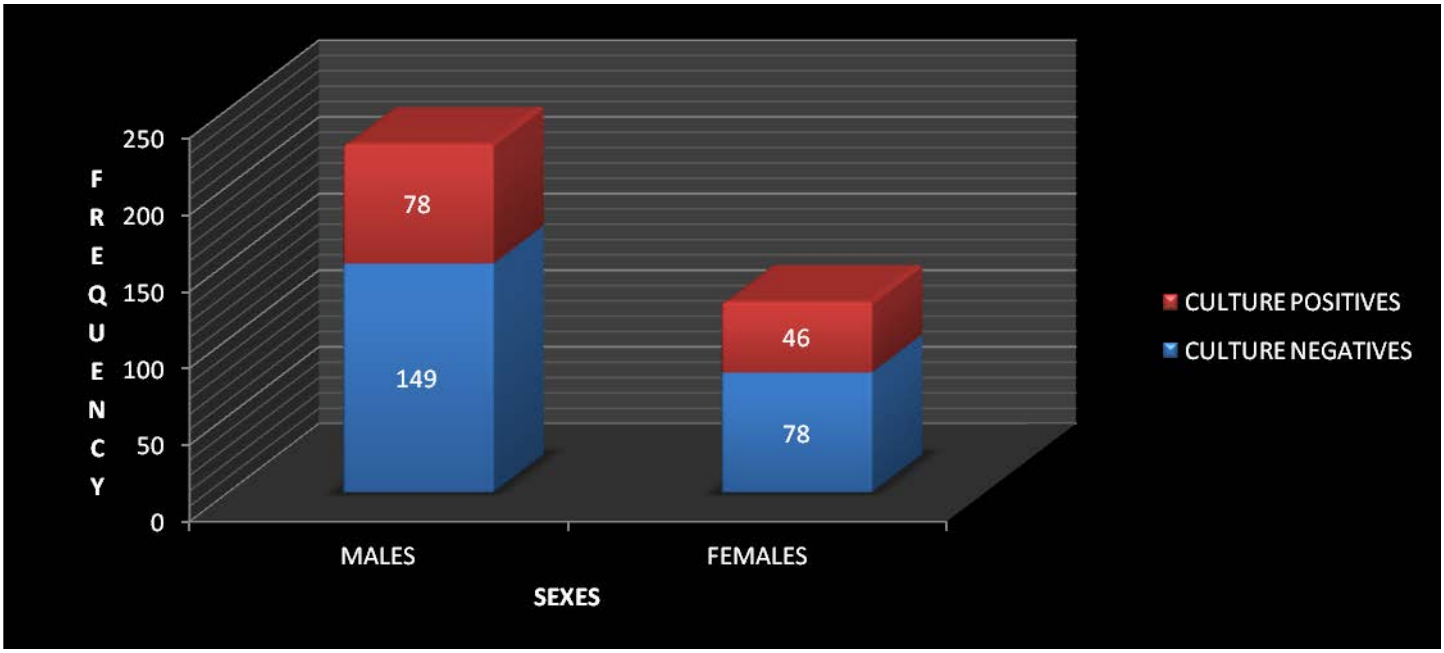

Figure 1: Distribution of Males & Females among Positive Blood Culture Samples

The mean age of the neonates was found to be 1.95 days and the neonatal age ranged from 1 day to 28days.

Among the 382 suspected cases of neonatal sepsis, culture positivity among the EONS and LONS cases were found to be 47.38% (n=59/124) and 52.42% (n=65/124) respectively and the association between the type of neonatal sepsis and blood culture positivity was found to be insignificant (p>0.05) as shown in Table 1.

Of the 124 isolates, Gram negative isolates (73) (58.87%) followed by Gram positive isolates (47) (37.9%) and *Candida albicans* (04) (3.23%).Amongst the Gram negative isolates the most common organisms were *Escherichia coli (*30) (41.09%) followed by *Klebsiella species* (23) (31.51%) while *Staphylococcus aureus* (24) (51.06%) and Coagulase negative *Staphylococci* (CONS) (14) (29.79%)were among the Gram positive isolates as depicted in Table 2 & 3.

Table 1: Distribution of Culture Positive Cases among EONS & LONS Cases.

S. No	Type of Neonatal Sepsis	Culture		Total	p value
		Negative	Positive		
1.	EONS	146 (71.22%)	59 (28.78%)	205 (100.00%)	0.122
2.	LONS	112	65	177 (100.00%)	

		(63.28%)	(36.72%)	
3.	**Total**	258 (67.54%)	124 (32.46%)	382 (100.00%)

χ^2 = **2.383 (using chi square corrected test)**

Table 2: Gram Positive Isolates Among Early Onset & Late Onset Neonatal Sepsis Cases.

S.NO	ETIOLOGICAL AGENTS	ISOLATED FROM EONS CASES (n=19)	ISOLATED FROM LONS CASES (n=28)	TOTAL	p value
1.	*Staphylococcus aureus*	11 (57.89%)	13 (46.43%)	24 (51.06%)	
2.	*CoNS*	01 (5.26%)	13 (46.43%)	14 (29.79%)	
3.	*Group B Streptococci*	05 (26.32%)	00 (0.00%)	05 (10.64%)	0.001
4.	*Enterococci*	02 (10.53%)	02 (7.14%)	04 (8.51%)	
	Total	19 (40.42%)	28 (59.57%)	47 (100%)	

Table 3: Gram Negative Isolates Among Early Onset & Late Onset Neonatal Sepsis Cases.

S.NO	ETIOLOGICAL AGENTS	ISOLATED FROM EONS CASES	ISOLATED FROM LONS CASES	TOTAL	p value
1.	*Escherichia coli*	22 (55.00%)	08 (24.24%)	30 (41.10%)	
2.	*Klebsiella species (K. oxytoca, K.pneumoniae)*	10 (25.00%) 04 06	13 (39.39%) 03 10	23 (31.51%) 07 16	
3.	*P.aeruginosa*	01 (2.50%)	07 (21.21%)	08 (10.96%)	
4.	*Proteus species (P.mirabilis, P.vulgaris)*	03 (7.50%) 02 01	03 (9.09%) 02 01	06 (8.22%) 04 02	0.02
5.	*Citrobacter species*	03 (7.50%)	01 (3.09%)	04 (5.48%)	
6.	*Acinetobacter*	01 (2.50%)	01 (3.09%)	02 (2.74%)	
	Total	40 (54.79%)	33 (45.21%)	73 (100%)	

ESBL Production: The isolates of *E.coli* and *Klebsiella* spp showing inhibition zone size of ≤ 22 mm with Ceftazidime (30 µg), ≤ 25 mm with Ceftriaxone (30 µg), and ≤ 27 mm with Cefotaxime (30 µg), ≤ 27 mm with Aztreonam (30 µg) were identified as presumptive ESBL producers. In the screening test, which involved detection of resistance to one or more of the four cephalosporin antibiotics (ceftazidime, cefotaxime, ceftriaxone and aztreonam), 29 (54.72%) were found to be resistant against the antibiotics. Of the 30 *E. coli* isolates, 16

(53.33%) were found to be resistant to one or more of the screening agents. Of the 23 *Klebsiella* isolates, 13(56.52%) were found to be resistant to one or more of the screening agents.

Prevalence of ESBL producing *Escherichia coli* and *Klebsiella* species among the neonatal cases of sepsis with these bacteria were found to be 45.28%. Individually the prevalence for *E. coli* and *Klebsiella* species were found to be 43.33% and 47.83%respectively.Screening test for presumptive ESBL producers detected29 (54.72%) isolates to be presumptive ESBL producers, *E. coli* (16, 53.33%), *Klebsiella species* (13, 56.52%). The positivity of confirmatory tests by combined disc diffusion test (CDDT), MIC test, double disk approximation and E strip test are tabulated in Table 4 and the validity of these tests considering CDDT as standard test as described by CLSI guidelines is depicted in Figure2.

Table 4: **Screening & Confirmatory Tests for ESBL Detection in Different Isolates**.

S.No	Name of the Tests	*E.coli(n=30)*	*Klebsiella(n=23)*	Total(n=53)
1.	Screening Test	16 (53.33%)	13 (56.52%)	29 (54.72%)
Confirmatory Tests				
2.	Combined Disc Diffusion Test	13 (43.33%)	11 (47.83%)	24 (45.28%)
3.	MIC Reduction Test	13 (43.33%)	11 (47.83%)	24 (45.28%)
4.	Double Disc Approximation Test for ESBL	9 (30.00%)	08 (34.78%)	17 (32.07%)
5.	Ezy MIC™ Strip Test	11 (36.67%)	09 (39.13%)	20 (37.73%)

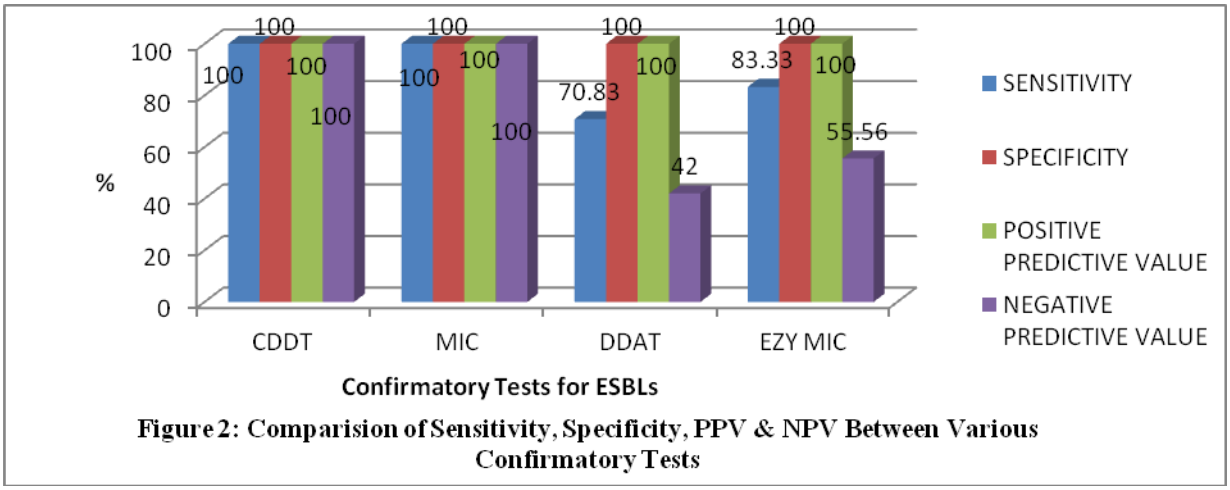

Figure 2: Comparision of Sensitivity, Specificity, PPV & NPV Between Various Confirmatory Tests

Discussion

Neonatal septicemia endures to be noteworthy reason for morbidity and mortality predominantly in developing countries where the diagnostic techniques are limited. Evaluation of tests for neonatal sepsis is important because the infection may present a very serious threat to the neonates as the clinical diagnosis of the cases of neonatal sepsis is onerous as it presents with nonspecific symptomatology. Multi resistant organisms like ESBL producing strains especially *Escherichia coli* and *Klebsiella species* have often been implicated with neonatal sepsis at tertiary care hospitals according to the past studies [10, 11]. The most common mechanism of antibiotic resistance among the Gram negative bacilli are

production of β-lactamase. Among the β-lactamases, the most common modes are the production of ESBLs and AmpCs. Resistance caused by AmpC β-lactamase is less common than the production of ESBLs, but may be more difficult to detect [12].

Blood culture positivity rate among neonates in the present study was found to be 32.46%. This result is in line with the rate of 31.5% reported by Khanna A et al [13].A wide variation in the positivity of blood culture has been delineated over the years from different centers from our country as well as across the globe. However a higher positivity of 55.8% was reported by Shukla O S et al from Vadodara, Gujarat [14]. The lower blood culture positivity might be due to slightly lesser number of risk factors associated with neonatal septicemia in the present study site. The higher rate of sepsis among various studies can be due to illiteracy among the patients, low socioeconomic condition around the study centre, poor antenatal care and high use of invasive procedures etc.

Of the 124 cases of blood culture positive cases, higher positivity of 34.36% were found in males and 29.67% in females. Male predominance is because of higher proportion of males in the study sample. No statistical significance ($p>0.005$) was attributed to the differences seen in distribution with respect to sex. A higher male to female ratio was also noted by Monica Lazarus et al 2018 in their study conducted in Madhya Pradesh [15].

The present study has shown a preponderance of Gram negative isolates (58.87%) as compared to Gram-positive isolates (37.9%) and yeast like fungi (3.22%). This is in line with the study conducted by Khanna et al in 2016 which reported 53.93 % of cases by Gram Negative isolates, 24.72% cases by Gram positive isolates, and 21.35% by yeast like fungi i.e. *Candida* species [13] while a contrasting percentage of 40% and 60% reported by Thakur et al in 2016 [16]. The most frequently isolated organism among Gram positive bacteria were *S. aureus* (n=24/47) (51.06%) which is comparable in EONS and LONS (EONS-57.89%, LONS-46.53%) followed by CoNS (29.79%) which were more common in LONS (46.43%) cases. This finding is comparable to the study conducted by Kumar R *et al* in Bihar [17] and Reddy KA et al from Telangana [18]. This is in line with the findings of Hasibuan B S et al in a referral center in Indonesia which reported isolation rate of *Staphylococcus aureus* as 5.1% and 4.0% among early onset and late onset neonatal sepsis, coagulase negative staphylococci (CoNS) were isolated in 6.33% and 14.67% among early onset and late onset neonatal sepsis cases respectively [19]. The colonization of the skin and nasopharynx by CONS and *S. aureus* in health care workers, the invasive procedures, lack of disinfection practice may lead to transmission of Gram positive organisms to neonates. Group B Streptococci (26.32%) were more common in EONS cases. This difference in distribution among various Gram positive bacteria was found to be statistically significant ($p<0.05$).

In the present study, *E. coli* species (41.09%) and *Klebsiella* species (31.51%) were most common Gram negative isolates which is similar to the findings reported in the National Neonatal Perinatal Database [20]. In contrast Khanna et al and Reddy KA et al in their study found the *Klebsiella* species as predominant pathogen (20.2 %) and (25%) followed by *Escherichia coli* (14.6 %) and (8.33%)[14, 18].

In the present study, 53.33% of *E. coli* and 56.52% of *Klebsiella* species were found to be positive for probable production of ESBLs in the screening test. The screening test involved ceftriaxone, cefotaxime, ceftazidime and aztreonam disks but not cefpodoxime. It was observed that each of the four cephalosporins were able to detect probable ESBL producers in>96.88% of isolates. This is

comparable to a study from Nepal, in which the screening test for presumptive ESBL producers detected100% of the isolates to be presumptive ESBL producers using the same four antibiotics [21].Of the 29*Escherichia coli* & *Klebsiella* isolates from the neonatal sepsis cases that were positive in the screening test in the present study, 24 (82.76%) isolates were phenotypically confirmed as ESBL producers by the CLSI phenotypic confirmatory method *i.e.* combined disc diffusion method and MIC reduction test. Detection of ESBL production among the isolates positive in the screening test has shown to range from 67.57% to 91.1% in the other studies [21, 22].Of the 16*E. coli* isolates that were primarily positive in the screening test, ESBL production was confirmed in 13 (81.25%) isolates. Likewise, of the 13*Klebsiella*isolates, 11 (84.61%) were confirmed as ESBL producers. Thus, from the total population (53) of *E.coli* and *Klebsiella* species included in the present study, ESBLs were detected in 13 (24.53%) *E. coli* and 11 (20.75%) *Klebsiella* isolates.

In this study, ESBL positive strains had been identified by screening test, and each is confirmed by combined disc diffusion test (CDDT), MIC test, E strip test and the double disk approximation test. For evaluation of various tests, Combined Disc Diffusion Test was considered reliable as stated by CLSI guidelines and was taken as standard. Other methods were compared with CDDT. ESBL production was confirmed by combined disc diffusion test in 45.28% isolates. Of the *E. coli* and *Klebsiella* isolates which were positive in screening test, 81.25% and 84.61%were phenotypically confirmed as ESBL producers each by CDDT and MIC test respectively, while 68.75% and 69.23% were confirmed by E strip test. The double disk approximation tests for ESBL among *E.coli* and *Klebsiella* isolates were positive in 56.25% and 61.53% of cases respectively. This is in line with the study by Shaikh et al who reported detection rate by MIC (agar diffusion) in 100% isolates, by combined disc diffusion test in 87.30% and Double disk synergy test in 77.78% [23].Similarly another study from Dhaka reported detection of ESBL by screening test to be 69.35% (n=86) while DDST confirmed53.49% (n=46) ESBL strains while E test determined 63.95%(n=55) ESBL producing isolates [24].

In the current study, the sensitivity and specificity of MIC reduction test was found to be 100%. This is comparable to the study conducted in Netherlands which reported the calculated sensitivity as 99.6%, and the specificity as 91.1% [25]. The slight difference can be due to the difference in sample size and methodology adopted for the study. It also justifies the use of MIC reduction test in addition to CDDT as confirmatory tests as per CLSI guidelines wherever possible and feasible.

Simultaneously in the present study the sensitivity, specificity, positive predictive value & negative predictive value of DDAT/DDST was found to be 70.83%, 100%, 100% and 42% respectively which is akin to the study from Bangladesh which observed the sensitivity, specificity, positive predictive value and negative predictive value of Double Disc Synergy Test (DDST) to be 83.1%, 100%, 100%, and 77.9% respectively [26]. Results of these studies and our study indicate that the DDST method is characterized by high sensitivity and specificity but the negative predictive value is low in our study as compared to others. Most significant advantage of DDST is that it is technically simple and the interpretation of the test is prejudiced. In this study the sensitivity of DDAT was 70.83%, and negative predictive value is very low, making it clear that it missed a fair number of ESBL negative strains.

By using the E-test in the present study the sensitivity, specificity, positive predictive value, negative predictive

value were found to be 83.33 %, 100%, 100% and 55.56% respectively. On the contrary a study in Nigeria using the E-test[MIC], a sensitivity of 100%, specificity of 99%, positive predictive value of 98% and negative predictive value of 100% were observed [27]. The results obtained by other researchers, and our own, indicate that the E-test has a higher sensitivity, in comparison to results obtained by DDAT.

Conclusion

Considering the limitations of various factors like validity, time of testing, expenses and challenging nature of the isolates, the four phenotypic methods were highly sensitive and specific for ESBL detection, comparing with the standard method of CDDT as stated by CLSI guidelines 2016, E-strip EZY[MIC] test was found to be more sensitive as compared to DDAT. The only limitation of the E strip test in developing countries is the high cost as compared to the antibiotic discs used in DDAT. The routine detection of ESBLs producing organisms by conventional methods should be carried out in every laboratory as the molecular methods are not possible to be performed.

References

1. Al-Shamahy HA, Sabrah AA, Al-Robasi AB, Naser SM. Types of bacteria associated with neonatal sepsis in al-Thawra university hospital, Sana'a, Yemen, and their antimicrobial profile. Sultan Qaboos Univ Med J. 2012; 12(1):48–54.
2. El-Din E M R S, El-Sokkary M M A, Bassiouny M R and Hassan R Epidemiology of neonatal sepsis and implicated pathogens: a study from Egypt. Biomed. Res. Int.2015;509484.
3. Weston EJ, Pondo T, Lewis MM, Martell-Cleary P, Morin C, Jewell B, Daily P, Apostol M, Petit S, Farley M, et al. The burden of invasive early-onset neonatal sepsis in the United States, 2005-2008. Pediatr Infect Dis J 2011; 30:937-41.
4. Emery CL. Weymouth LA. Detection and Clinical Significance of Extended – Spectrum beta-Lactamases in a Tertiary care Medical Center. J Clin Microbiol. 1997;35:2061–7.
5. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-fifth Informational Supplement M100-S25. Wayne, PA, USA: CLSI; 2015.
6. Paterson DL. and Robert A. Bonomo.Extended-Spectrum -Lactamases: a Clinical Update Clinical Microbiology Reviews. 2005;18 (4): 657-686.
7. European Society of Clinical Microbiology and Infectious diseases, EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance:EUCAST;2012.
8. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-first Informational Supplement M100-S20. Wayne, PA, USA: CLSI; 2010.
9. arlier V, Nicolas M, Fournier G, Phillipon A. ESBLs conferring transferable resistance to newer β- lactam agents in *Enterobacteriaceae*; hospital prevalence and susceptibility patterns. Rev Infect Dis. 1988;10:867–78.
10. sai M-H, Lee I-T, Chu S-M, Lien R, Huang H-R, Chiang M-C, et al. Clinical and Molecular Characteristics of Neonatal Extended-Spectrum βLactamase-Producing Gram-Negative Bacteremia: A 12-Year Case-Control-Control Study of a Referral Center in Taiwan. PLoS ONE. 2016;11(8):e0159744.

11. Sakellariou C, Gürntke S, Steinmetz I, Kohler C, Pfeifer Y, Gastmeier P, et al. Sepsis Caused by Extended-Spectrum Beta-Lactamase (ESBL)-Positive K. pneumoniae and E. coli: Comparison of Severity of Sepsis, Delay of Anti- Infective Therapy and ESBL Genotype. PLoS ONE. 2016;11(7):e0158039.
12. Hsieh, W.S., Wang, N.Y., Feng, J.A., Weng, L.C., Wu, H.H. Identification of DHA-23, a novel plasmid-mediated and inducible AmpC beta-lactamase from *Enterobacteriaceae* in Northern Taiwan. *Front Microbiol.* 2015;6: 436.
13. Khanna A, Khanna M, Gill M. ESBL Producing Gram Negative Bacteria-A Cause of Concern in Neonatal Septicemia in a Tertiary Care Hospital. *Int.J.Curr.Microbiol.App.Sci.* 2016;5(10): 807-813.
14. Shukla OS, Rawat A. Clinical profile and outcome of early onset sepsis in high risk very low birth weight neonates. Int J Contemp Pediatr 2018;5:389-94.
15. Lazarus Monica, J. Seth Riti, Kinnare Amit B. Role of Sepsis Screen Parameters in Early Diagnosis of Neonatal Septicemia. Int.J.Curr.Microbiol.App.Sci. 2018;7(01): 2410-2419.
16. Thakur S, Thakur K, Sood A, Chaudhary S. Bacteriological profile and antibiotic sensitivity pattern of neonatal septicaemia in a rural tertiary care hospital in North India. IJMM. 2016; 34(1): 67-71.
17. Kumar R, Kumari A, Kumari A, Verma N. Evaluation of perinatal factors in neonatal sepsis at tertiary centre. Int J Reprod Contracept Obstet Gynecol 2017;6:4981-5.
18. Reddy KA, Kanth SU. Bacteriological profile and antibiotic sensitivity patterns of blood cultures. Int J Contemp Pediatr 2016;3:1221-6.
19. Hasibuan B S. Comparison of microbial pattern in early and late onset neonatal sepsis in referral center Haji Adam Malik hospital Medan Indonesia.IOP Conf. Series: Earth and Environmental Science. 2018;125:012053.
20. National neonatal perinatal database-WHO Newborn CC. Available at http://www.newbornwhocc.org/pdf/nnpd_ report_2002-03.PDF. [Last accessed on 2017 Aug 17].
21. Maharjan A, Bhetwal A, Shakya S. Ugly bugs in healthy guts! Carriage of multidrug-resistant and ESBL-producing commensal Enterobacteriaceae in the intestine of healthy Nepalese adults. Infection and Drug Resistance 2018:11 547–554.
22. Peirovifar A, Rezaee MA, Gharehbaghi MM. Prevalence of Multidrug Resistant Extended-Spectrum Beta-Lactamase Producing Gram-Negative Bacteria in Neonatal Sepsis. Int J Women's Health Reproduction Sci 2014;2(3): 138-145.
23. Shaikh N. K, Mundhada S. G., Lalngaihzuali R., Ingole K. V.Comparison of different phenotypic methods for the detection of extended spectrum β-lactamase (ESBL) in bacterial isolates from tertiary care centre.Int J Cur Res Rev. 2016;8 (11):10-14.
24. Paul S, Jhora S.T., Dey P.P. Evaluation of Phenotypic Methods to Identify Extended Spectrum Beta-lactamase (ESBL) Producing Gram negative Bacteria. Bangladesh J Med Microbiol. 2014; 8(1):21-24.
25. Overdevest I.T., Willemsen I., Elberts S., Verhulst C., Kluytmans J.A.: Laboratory detection of extended-spectrum-beta-lactamase- -producing Enterobacteriaceae: evaluation of two screening Agar plates and two confirmation techniques. J. Clin. Microbiol., 2011; 49: 519-522.
26. Chowdhury AHMSK, Nandi S, Rahman M, Karim ASMA, Mamtaz SSH. Ara NR, Sultana S. Comparison between Phenotypic Confirmatory Test & Double Disc Synergy Test in Detection of

Extended Spectrum β-Lactamases Producers Among Gram-Negative Bacilli. Chattagram Maa-O-Shishu Hospital Medical College Journal. 2016;15(2):3-8.

27. Yahaya M, Bala GG, Bello ZS et al. Evaluation of two phenotypic methods for the confirmatory testing of extended spectrum beta lactamases producing strains of Klebsiella pneumoniae Int J Health Sci Res. 2017; 7(4):113-118.