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Detection of ESBL Production Among Hospital AndCommunity Isolates of Klebsiellapneumoniae

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Abstract: *Extended spectrum β-lactamases (ESBLs) are a group of enzymes found in certain species of Gram-negative bacilli notably Klebsiella and Escherichia coli and confer upon the bacteria ability to hydrolyse β-lactam rings of third generation cephalosporins and/or aztreonam. Bacteria produce ESBLsdue to mutation of TEM-1, TEM-2 and SHV-1. Widespread use of third generation cephalosporins causes mutations leading to emergence of ESBLs.Because of extended substrate range, they are called Extended Spectrum β-lactamases.Initially restricted to hospital acquired infections(HAI), ESBL-producing bacteria are emerging as major pathogens causing outbreaks,Klebsiella pneumonia being the commonest in hospital environment.Present study evaluated the techniques of detection of ESBLs in Klebsiellapneumoniae isolates from clinical samples like wound-pus, blood, urine, stool and sputum ofin and outpatients ofMahathma Gandhi Memorial Hospital, Warangal, Telangana State. Among various methods available to detect ESBLs, techniques selected to study were double disc synergy test (DDST)and phenotypic confirmatory disc diffusion Test (PCDDT).Among 200 clinical isolates tested, 72 were resistant to 3rd generation cephalosporins (3GCs) which were subjected to DDST and PCDDT. Among 72, 34 were positive for ESBL with DDST (47.22%) and 36 with PCDDT (50.00%). PCDDT was more sensitive than DDST in ESBL detection.*

Keywords: *Double disc synergy test (DDST), Extended spectrum β-lactamases (ESBLs), Hospital associated infections (HAI),Phenotypic confirmatory disc diffusion test (PCDDT), Third generation cephalosporins (3GCs).*

I. Introduction

Extended spectrum β-lactamases (ESBLs) are a group of enzymes found in certain species of Gram-negative bacilli notably Klebsiella and Escherichia coli. These enzymes confer upon the bacteria the ability to hydrolyse the β-lactam rings of third generation cephalosporins and/or aztreonam [1].Because of extended substrate range, they are called Extended Spectrum β-lactamases [2]. ESBL producing Klebsiellapneumoniaeare being increasingly reported in the hospital environment in the past decade due to extensive use of newer generation cephalosporins. ESBLs are produced by bacteria due to mutation of TEM-1, TEM-2 and SHV-1 genes. Widespread and injudicious use of third generation cephalosporins and/or aztreonam is the major cause of mutations leading to emergence of ESBLs [3]. The genes which code for ESBL production are located on large conjugated plasmids of 80-160 kb in size[4]. Organisms carrying these genes exhibit resistance to a variety of classes of antibiotics [5, 6] like trimethoprim, amikacin, streptomycin, gentamicin etc. [7]. Initially restricted to hospital associated infections (HAI), the ESBL-producing bacteria are emerging as major pathogens causing outbreaks [8, 9]. Since Klebsiellapneumoniae is the commonest organism inhabiting the hospital, and the commonest organism acquiring resistance through conjugation, it is imperative to assess the prevalence of ESBL producing Klebsiellapneumoniae to that major outbreaks can be averted after detection and elimination of sources in the hospital, which is important for epidemiological reasons. The present study was aimed at detecting ESBLs among 200 clinical isolates of Klebsiellapneumoniae from both in and outpatients suffering from post-operative and burn-wound infections, septicaemia, lower respiratory tract infections, urinary tract infections and diarrhoea.

II. Material And Methods

The material for the present study consisted of two hundred clinical isolates of Klebsiellapneumoniae. These strains were isolated from various clinical samples e.g. sputum, pus, urine, stool and blood. The patients were either out-patients or in-patients admitted into MGM Hospital, Warangal in various units like medical,

surgical, orthopaedics, burns, infectious diseases wards, paediatric and intensive care units. Hundred isolates were from the out-patients and a hundred from in-patients. The strains of Klebsiellapneumoniae were identified as per the standard guidelines [10]. (Fig.1, 2).The antibiograms of all the isolates were studied with a set of eight antibiotic discs consisting of amoxycillin (30 µg), gentamicin (10µg), amikacin (30 µg), ciprofloxacin (30 µg), cefuroxime (30 µg), ceftazidime (30 µg), cefotaxime (30 µg) and ceftriaxone (30 µg). Kirby-Bauer's method of disc diffusion susceptibility testing was done as per the NCCLS guidelines. (Fig. 3,4). The isolates were categorised into two groups based on their susceptibility or resistance to 3GCs. Group I consisted of sensitive strains which have shown a zone diameter of more than 17 mm for all the 3GCs. Group II were resistant strains which have shown a zone diameter of less than 17 mm for any one of the 3GCs [11]. (Fig. 5).Strains belonging to the second group were tested for ESBL production by two methods, double disc synergy test [12] and phenotypic confirmatory disc diffusion test [13].The control strains used in the present study were Klebsiellapneumoniae ATCC 700603 as positive control, Escherichia coli ATCC 25922 as negative control and in-house control.

2.1 Double disc synergy test (DDST) [12]:DDST determines synergy between a disc of augmentin (amoxycillin 20µg and clavulanic acid 10µg) and 30µg disc of each 3GCs (ceftazidime, ceftriaxone and cefotaxime) [9, 14]. Mueller Hinton agar plates were prepared and inoculated with standardised inoculum (0.5 McFarland tube) to form a lawn culture. 30µg discs each of 3GCs was placed on the agar at a distance of 15 mm centre to centre from augmentin disc. ESBL production was interpreted if the inhibition zone round the test antibiotic disc increased towards the augmentin disc, or if neither discs were inhibitory alone but bacterial growth is inhibited where the two antibiotics diffused together [15]. The diameter of the zone of inhibition for each antibiotic is measured and interpreted as resistant, intermediate susceptible or susceptible according to NCCLS criteria [16]. The strains which showed widening of the zone of inhibition around a 3GC disc of more than 3 mm towards the augmentin disc were considered as ESBL producers by DDST [15]. (Fig. 6, 7, 8).

2.2 Phenotypic confirmatory disc diffusion test (PCDDT) [13]: PCDDT requires use of both cefotaxime (30µg) and ceftazidime discs alone and in combination with clavulanic acid (30 µg). Discs of ceftazidime and cefotaxime with clavulanic acid (30µg/10 µg) were prepared using a stock solution of clavulanic acid at 1,000 µg/ml (taken from a small aliquot kept frozen at -70°C).10µl of clavulanic acid solution was added to these discs within an hour before these were applied to the plates. An increase in the zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was observed. For ceftazidime,an increase in zone diameter of$\geq$5 mm and for ceftriaxone,$\geq$ 3 mm was considered as an ESBL producer [15, 16]. (Fig. 9).

III. Results

Among the three cephalosporins namely ceftaziime, ceftriaxone and cefotaxime used in the disc diffusion sensitivity testing, ceftazidime gave maximum number of resistant strains compared to the other two.(TABLE I).It is observed that some of the strains showed susceptibility to one 3GC disc while at the same time showing resistance to the other. It is the personal experience of the authors that a minimum of three discs of 3GCs have to be initially employed to pick up resistant strains for ESBL testing.As many as 72 isolates of Klebsiellapneumoniae (60 from hospital and 12 from community) have shown resistance to 3GCs. (TABLE II). Considering the disease burden of more than 2,000 out-patients and more than 800 in-patients per day in the Mahatma Gandhi Memorial Hospital, Warangal, the resistance percentage of Klebsiellapneumoniaeidentified for the first time is a matter of serious concern necessitating urgent interventional measures.Among the 60 resistant isolates of Klebsiellapneumoniaefrom the hospital, 28 demonstrated ESBL production by DDST whereas PCDDT detected 30 strains. (TABLEIII).

The results of the present study correlated well with Shukla et al, 2004 [14]. Elsewhere in India the percentages of ESBL producing Klebsiellapneumoniae showed a range of 6 to 64%.(TABLE IV). The figures are likely to scale up in future if the use of 3GCs is not restricted for empirical treatment of critically ill conditions alone.DDST was to detect only 34 strains (47.22%) from among the 72 tested whereas PCDDT detected 36 ESBL strains (50%) with an increase of zone diameter more than 5 mm around a ceftazidime + clavulanic acid disc when compared to ceftzidime disc alone.

IV. Discussion And Conclusion

According to Lalitha, (2005), the choice of which 3GC to be tested in DDST was critical. For e.g. one enzyme may actively hydrolyse ceftazidime but may have poor activity on cefotaxime. According to her, the sensitivityof screening for ESBLs for enteric organisms improved with the use of more than 1 of the 5 antimicrobial agents suggested (cefpodoxime, ceftazidime, aztreonam, ceftotaxime and ceftriaxone). She has suggested cefpodoxime and ceftriaxone for screening by DDST as they showed the highest sensitivity for ESBL detection [17]. In the present study three antibiotics belonging to 3GCs were used. We have observed that the

production of ESBL is best demonstrated at a distance of 15 mm centre to centre.As per the NCCLS guidelines an inhibition zone diameter of 0-14 mm is considered resistance and a diameter of 15-17 mm is considered intermediate [16]. Among the 34 ESBL producers detected by DDST, 24 showed a zone below 14 mm (70.58%). The rest of the isolates showed zones of intermediate sensitivity. It was thus observed that isolates showing zones of intermediate sensitivity were also potential ESBL producers.

In the present study PCDDT has detected 36 ESBL producers whereas DDST detected 34 (sensitivity of 94.44% by DDST compared to 100% by PCDDT). (Fig. 10).The strains which were 3GC resistant and negative by DDST and PCDDT (non-ESBL producers) could not be tested for Amp Cβ-lactamase production due to non-availability of cefoxitin discs at the time of study. (Fig. 11).According to Singhal et al, (2005), ESBL producers can be isolated both from hospital (in-patient units) as well as from community (out-patient clinics), whereas Amp C harbouring organisms are found only in admitted patients [18]. It has been reported that at present in India, Amp C harbouring isolates are largely restricted to the hospitalised patients alone [19].

The plasmid mediated ESBL and Amp C producing strains can become resistant to cephamycins, oxyimino β-lactams and carbapenem, due to loss of an outer membrane porin protein. Loss of porins can also augment resistance provided by ESBLs, as indicated by increase in MIC (minimum inhibitory concentration) to3GCs. More extensive study in relation to OMP profiles to resistance patterns is recommended to emphasise clinical impact of porin-mediated β-lactam resistance in India [20].

Table I
Individual Resistance Pattern of Test Isolates to 3GCs

No. of the isolates tested	Name of the Cephalosporin	Hospital isolates				Community isolates			
		Susceptible		Resistance		Susceptible		Resistance	
		No.	%	No.	%	No.	%	No.	%
200 (100 Hospital and 100 Community)	Ceftazidime	40	40%	60	60%	88	88%	12	12%
	Ceftriaxone	54	54%	46	46%	90	90%	10	10%
	Cefotaxime	56	56%	44	44%	90	90%	10	10%

Table II
Resistance to 3GCs in the Test Isolates

Source	No. of test samples	Resistant to 3GCs	
		No.	Percentage
Hospital	100	60	60%
Community	100	12	12%

Table III
Percentage Positivity of ESBLs – Comparison of DDSTand PCDDT

DDST				PCDDT			
ESBL Positive		ESBL Negative		ESBL Positive		ESBL Negative	
No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage
28 (Hospital)	47%	32	53%	30	50%	30	50%
6(Community)	50%	6	50%	6	50%	6	50%

Table IV
Percentage of ESBLs in Different Study Groups in India

S. No.	Year	Place	Author	No. studied	No. ESBL	Percentage
1	1997	Delhi	Revathi	100	53	53.00%
2	2002	Chennai	Subha and Ananthan	120	8	6.66%
3	2004	Aligarh, U.P.	Shukla et al	120	32 (by PCDDT) 29 (by DDST)	26.66% 24.16%
4	2005	Haryana	Singhal et al	272	173	63.60%
5	2006	Chennai	Menon et al	70	14	20.00%

Fig. 1 Lactose fermenting mucoid colonies of Klebsiellapneumoniae on MacCconkey's agar

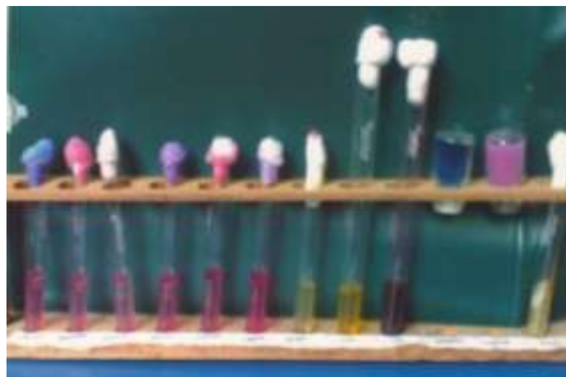

Fig.2 Biochemical reactions of Klebsiellapneumoniae

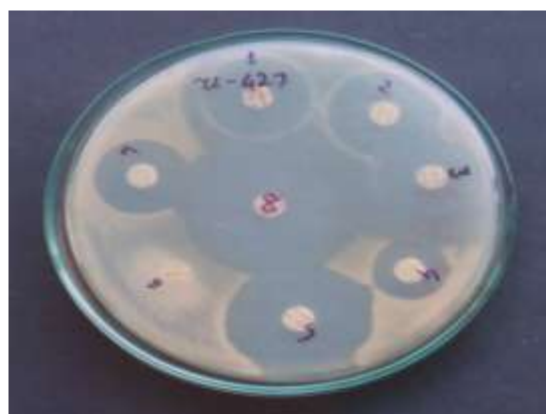

Fig. 3 Antibiogram of the isolate showing absence of inhibition zone around ceftazidime disc

Fig. 4 Demonstration of 3GC resistant isolate of Klebsiellapneumoniae

Fig. 5Resistance to all three of the 3GCs

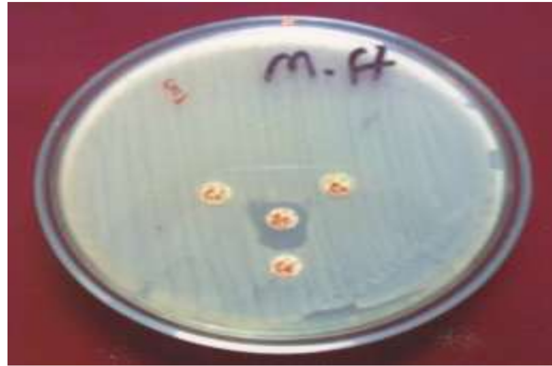

Fig. 6ESBL production:DDST positive with ceftriaxone alone

Fig. 7 ESBL production against 2 of the3GCs: cefotaxime and ceftriaxone

Fig. 8ESBLproduction detected by DDST

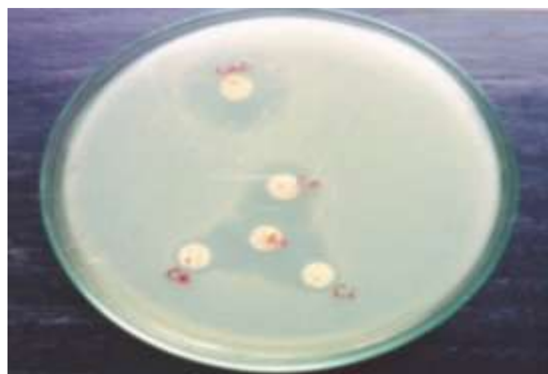

Fig. 9   Demonstration of ESBL production by DDST and PCDDT on the same plate

Fig. 10 DDST negative and PCDDT positive

Fig. 11 DDST and PCDDT negative

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