Original Article

Antimicrobial Susceptibility Testing and Esbl Detection from the Bacteria in Haemodialysis Patients

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ABSTRACT

Objective of Study: To find out the antimicrobial sensitivity and extended spectrum β-lactamase producing organisms among clinical isolates recovered from patients on haemodialysis. Extended spectrum β-lactamase are enzymes produced from some strains of gram negative bacilli that mediate resistance to extended spectrum cephalosporin and aztreonam. They are most common in E.coli and Klebsiella species but are present in variety of enterobacteriacea.

Design of Study: Experimental and observational study.

Place and duration of Study: This study was carried out in Microbiology Department of BMSI, JPMC, Karachi. This study was carried out from June 2005 to June 2006.

Materials and Methods: A total of 250 cases irrespective of age and gender were included in this study. A total of 15 gram positive cocci (7.5%) and 175 (87.5%) gram negative organisms were recovered. In this study 66.66% E.coli and 33.33% Klebsiella species were ESBL producing. Antimicrobial sensitivity pattern in this study show that most of the organisms were sensitive to 2nd and 3rd generation cephalosporins and fluroquinolones i.e. ciprofloxacin.

Results: Table 1 shows isolation of ESBL producing organisms from 200 positive cases. Out of these 175 cases were from Enterobacteriaceae, among these 06 (3.42%) cases have been found to be ESBL producing organisms.

Conclusion: The results of this study support the use of initial antimicrobial therapy to reduce the spread of infection and other complications. Currently ciprofloxacin is regarded as the drug of choice for the treatment of infection caused by both gram negative and gram positive bacteria in patients on hemodialysis

Key words: Extended spectrum β-lactamase (ESBL), antimicrobial sensitivity.

INTRODUCTION

Bacterial infections of all types seem to be increased in incidence, but there is a particular risk of infections related to vascular access sites or devices in patients on haemodialysis. Many of these infections are due to sepsis, primarily arising from the vascular access site. Septicemia alone accounts for almost 11% of mortality in hemodialysis patients. Hemodialysis patients are also a sentinel population for the emergence of antimicrobial resistance, especially with regards to gram positive cocci (vancomycin resistant enterococci (VRE), methicillin resistant Staphylococcus aureus (MRSA), Staphylococcus aureus with reduced susceptibility to vancomycin intermediate Staphylococcus aureus (VRSA), and methicillin resistant Staphylococcus aureus (MRSA)[1].

Antimicrobial use in concern with patient to patient transmission of resistant strain, has caused a rapid increase in prevalence of antimicrobial resistance in recent years. This particularly includes threat to dialysis patients who have often been at the fore front of epidemic of resistance[2].

Resistance to Beta lactam antimicrobial agents especially Extended spectrum cephalosporin and other agents among clinical isolates of gram negative bacteria is on the rise worldwide. The antimicrobial resistant pathogens include Extended spectrum cephalosporin resistant E.coli, Klebsiella pneumoniae, Enterobacter Coloacae, Serratia and Citrobacter friundii and Pseudononas aeruginosa and Acinitobacter banmnni[3].

The emergence of extended spectrum β-lactamase (ESBL) producing Enterobacteriaceae, particularly Escherichia coli and Klebsiella pneumoniae, presents significant diagnostic and therapeutic challenges to the management of infections due to these organisms[4].

MATERIALS AND METHODS

A total of 190 bacteria were recovered from intravenous catheter tips from patients admitted in different hospitals e.g. Nephrology unit of JPMC, Kidney centre and SIUT. The Samples received initially incubated on blood agar, MacConkey agar and Chocolate agar. The samples were incubated at 37ºC for 24 hours. The organisms were primarily identified by standard techniques. The antimicrobial sensitivity test done by standard National Clinical Committee Laboratory Standard (NCCLS) method **(**Linscott and Brown 2005**)[5].**

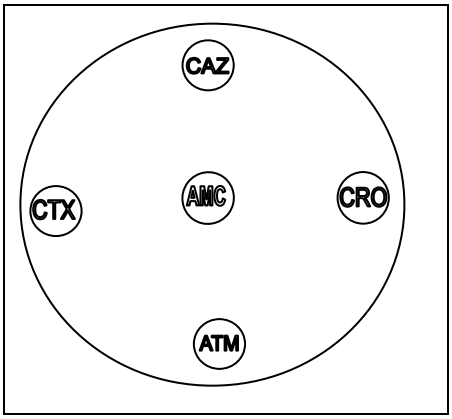
Extended spectrum beta-lactamase (ESBL) production was carried out by double disc diffusion method[6].
According to this method a susceptibility disc containing (Augmentin) Amoxicillin 10 µg+ Clavulanat acid 20 µg was placed in the center of plate containing Muller Hinton Agar. Amoxicillin 10 µg+ Clavulanat acid 20 µg (Augmentin) disc was placed as an inhibitor of beta-lactamase. Cefotaxime, Ceftazidim, Ceftriaxone and Azotreonam (30µg) each were placed at a distance of 30mm (center to center) from Amoxicillin 10 µg+ Clavulanat acid 20 µg (Augmentin).
Enhancement of inhibtion zone towards Amoxil + clavulanic acid disc indicating synergy of Clavulanic acid with anyone of the test antibiotics was taken as presumptive evidence of ESBL production. The quality control strains used for DDT or DDST testing were E-coli ATCC 25922 and Klebsiella pneumoniae ATCC 700603[7].

The following discs are applied on Muller Hinto Agar (MHA)

Disc	Concentra-tions	Oxoid disc code	Inhibition zone
Amoxil + clavulanic acid	20+10µg (C.A+Amp)		≥20
Ceftazidime	30µg	CAZ30	≤27
Cefotaxime	30µg	CTX30	≤27
Ceftriaxone	30µg	CRO30	≤25
Azactum	30µg	ATM30	≤27

Interpretation Results:
An organism was interpreted as containing an ESBL if there is an increase or extension of >5mm in the inhibition in the area between the Amoxicillin 10 µg+ Clavulanat acid 20 µg (Augmentin) disc and any one of the four cephalosporin discs in comparison with the zone of inhibition on the for side of that disc[6].

Muller Hinton Agar Plate With Antimicrobial Disc



- Diagram showing double disc diffusion (synergy) test used to detect the (ESBLs) Extended Spectrum Beta-Lactamase producing organisms (Jarliar et al., 1988) [4].

CAZ = Ceftazidime, CTX = Cefotaxime
AMC = Amoxicillin 10 µg+ Clavulanat acid 20 µg
CRO = Ceftriaxone, ATM = Aztreonum

RESULTS

Table 1 shows isolation of ESBL producing organisms from 200 positive cases. Out of these 175 cases were from Enterobacteriaceae, among these 06 (3.42%) cases have been found to be ESBL producing organisms.

DISCUSSION

A total of 250 subjects were selected and infection was positive in 200 (80%) cases where as 50 (20%) were negative. Same type of study has been done by Shaikh et al. (2005). Oncu et al. (2003) isolated only 3% fungus.
In present study antimicrobial sensitivity of isolated organisms has also been carried out. *E.coli* is highly sensitive with cephalaxin (80%), Ciprofloxacin (CIP) (86%), Ofloxacin (OFL) (88.9%), Amoxil + clavulanic acid (AMC) (77.9%), Cephazolin (CL) (84.5%), Cefuroxime (CXM) (64%) while highly resistant to Amoxicillin (AML) (72%), Neomycin (NEO) (79.50%), Gentamicin (GM) (66.7%) and ceftriaxone (CRO) (75.5%).
In this study the extended spectrum of beta lactamase (ESBL) producing organisms, showed resistance from 2nd and 3rd generation cephalosporin and carbapenum. ESBl production was mostly observed in E.coli 4 cases (66.66%) and Klebsiella species 2 cases (33.33%). Study done by Qadir (2005) also observed ESBl production in E.coli, Klebsiella and Pseudomonas aeruginosa.

CONCLUSION

The results of this study support the use of initial antimicrobial therapy to reduce the spread of infection and other complications. Currently ciprofloxacin is regarded as the drug of choice for the treatment of infection
caused by both gram negative and gram positive bacteria in patients on hemodialysis.

Table No. 1: Distribution of Esbl Producing Isolates

No. of specimens	No. of +ve organisms isolated	% of +ve isolates	No. of +ve organisms of Enterobacteriaceae family	No. of ESBL producing organisms	% of ESBL producing organisms
250	200	80%	175	06	3.42%

Table No. 2: Antimicrobial Sensitivity of Gram Negative Organisms Isolated From Hemodialysis Patients

Micro-organism Susceptibility	**AML 10µg**	**AMC 30µg**	**CZ 30µg**	**CL 30µg**	**CXM 30µg**	**CRO 30µg**	**GM 10µg**	**NEO 30µg**	**CIP 5µg**	**OFL 10µg**
E.coli										
Sensitive (%)	28.00	77.90	80.00	84.50	64.00	24.50	33.30	20.50	86.00	88.90
Resistant (%)	72.00	22.10	20.00	15.50	36.00	75.50	66.70	79.50	14.00	22.10
Klebsiella										
Sensitive (%)	29.50	74.50	75.00	70.50	63.50	66.00	35.00	22.20	88.50	73.50
Resistant (%)	70.50	25.50	25.00	29.50	36.50	34.00	65.00	77.80	11.50	26.50
Pseudomonas										
Sensitive (%)	12.00	35.50	64.50	65.50	62.00	69.00	18.00	22.50	78.50	79.00
Resistant (%)	88.00	64.50	35.50	34.50	38.50	31.00	82.00	77.50	21.50	21.00
Proteus										
Sensitive (%)	8.00	75.00	64.80	69.00	68.22	62.00	9.00	7.00	76.00	80.50
Resistant (%)	92.00	25.00	35.20	31.00	31.88	38.00	91.00	93.00	24.00	19.50

Key: Name with Abbreviation: Amoxicillin (AML), Amoxil + clavulanic acid (AMC), Cephalexin (CZ), Cephazolin (CL), Cefuroxime (CXM), Ceftriaxone (CRO), Gentamicin (GM), Neomycin (NEO), Ciprofloxacin (CIP), Ofloxacin (OFL)

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