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MOLECULAR EPIDEMIOLOGY AND CLINICAL OUTCOMES OF MULTIDRUG RESISTANT GRAM NEGATIVE BLOOD STREAM INFECTIONS AT A TERTIARY CARE HOSPITAL IN TELANGANA.

Clinical Microbiology

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ABSTRACT

Background: Multidrug resistance in the form of extended spectrum betalactamases (ESBL) and carbapenamases significantly hinders the treatment outcomes among gram negative blood stream infections(BSI). A study to evaluate the molecular profile and resistance patterns of gram-negative blood stream isolates was conducted in a tertiary care centre in South India. **Methods:** Gram negative clinical isolates from BSI over a period of six months were collected. Phenotypically identified ESBL and cabapenamase producers were tested for cabapenamase encoding genes (blaKPC, blaVIM, blaIMP, blaNDM, blaOXA-48, blaOXA-23,blaGES ; ESBL genes -blaCTX, blaTEM and blaSHV and colistin resistance determinants - mcr-1 and mcr-2 by PCR. Clinical and demographic data were compared with similar number of patients with non MDR isolates. **Results:**Of the 35 MDR bacteraemia patients tested in MDR group, blaOXA-48 and blaNDM were detected in 87.5% (21) and 75% (18) of 24 cabapenamase positive patients respectively. blaCTX-M7 and blaCTX-M19 were detected in 6/8 and 4/8 ESBL respectively and one patient with pan drug resistance had blaOXA-48, blaNDMcabapenamase genes and blaCTX-M7 ESBL. Risk factors for MDR bacteraemia were Klebsiellapneumoniaeinfection, secondary BSI, mechanical ventilation, malignancy, and treatment in an intensive care unit. Mortality was significantly more in patients with MDR bacteraemia (p=0.0125).**Conclusion:** blaOXA-48, blaNDMcarbapenamases and blaCTX-M7, blaCTX-M19 ESBL's were most commonly associated with gram negative MDR bacteraemia in present study with Klebsiellapneumoniaeinfection, secondary BSI, mechanical ventilation, malignancy, and treatment in an intensive care unit being significant risk factors.

KEYWORDS

bacteremia, carbapenemase, ESBL, Risk factors

INTRODUCTION

Blood stream infections (BSI) are a major cause of morbidity and mortality due to infectious diseases.[1-6] This is compounded by increasing antibiotic resistance particularly among gram negative bacteria. Plasmid borne beta lactamase enzymes have become widespread due to antibiotic overuse and extended spectrum beta lactamases (ESBL) are now common in both community and hospital settings. [7] Treatment of ESBL producing Enterobacterales with carbapenems has led to further increase in carbapenem resistance. Pan drug resistance including Colistin resistance has been reported in a few studies. [8] Increase in multidrug and pan drug resistance among gram negative isolates leads to limited therapeutic options and search for novel antibiotic combinations. Hence detection and reporting of resistance mechanisms is important for optimum clinical management. As cabapenamase production is associated with only slight increase in minimum inhibitory concentrations (MIC), molecular tests are useful over phenotypic methods for their detection. [9]

MATERIALS AND METHODS

A prospective observational study was conducted for six months from June 2019 to December 2019. Patients with blood stream infection (BSI) due to gram negative bacilli with signs and symptoms of infection were included in the study. Medical records were reviewed, and data was collected regarding patient characteristics, underlying illnesses, patients' location at onset of bacteraemia, treatment and mortality.

The blood cultures were performed on BacT/ALERT 3D (bioMeriéux-France) and microbial identification was done by VITEK 2 (bioMeriéux France). Gram negative isolates identified by VITEK 2 as carbapenamase, ESBL phenotype or colistin intermediate susceptible were further evaluated by molecular methods. The multidrug resistance phenotype was defined using the consensus definitions proposed by Magiorakos et al. [10]

DNA extraction was done by CTAB (cetyltrimethylammonium bromide) method. [11]The cabapenamase encoding genes (bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{NDM}, bla_{OXA-48}, bla_{OXA-23}, bla_{GES}; ESBL genes -bla_{CTX}, bla_{TEM} and bla_{SHV} and Colistin resistance determinants – mcr-1 and mcr-2 were confirmed by PCR, using the primers described below – table1

Table1: Oligonucleotides Used In This Study

Primer	Sequence	Gene	Product size (bp)
KPC-F	ATGTCACTGTATCGCCGTCT	bla_{KPC}	893
KPC-R	TTTTCAGAGCCTTACTGCCC		
VIM-F	GGTGTTTGGTCGCATATCGC	bla_{VIM}	504
VIM-R	CCATTCAGCCAGATCGGCATC		
IMP-F	CATGGTTTGGTGGTTCTTGT	bla_{IMP}	448
IMP-R	ATAATTTGGCGGACTTTGGC		
NDM-F	GGTTTGGCGATCTGGTTTTC	bla_{NDM}	621
NDM-R	CGGAATGGCTCATCACGATC		
OXA-48-F	ACGGGCGAACCAAGCATTTTTAC	bla_{OXA-48}	596
OXA-48-R	TGAGCACTTCTTTTGTGATGGCT		
OXA-23-F	CAGAATATGTGCCAGCCTC	bla_{OXA-23}	546
OXA-23-R	GCATTTCTGACCGCATTTCC		
GES-F	GCTTCATTCACGCACTATT	bla_{GES}	323
GES-R	CGATGCTAGAAACCGCTC		
MCR-1-F	AGTCCGTTTGTTCTTGTGGC	MCR-1	320
MCR-1-R	AGATCCTTGGTCTCGGCTTG		
MCR-2-F	CAAGTGTGTTGGTCGCAGTT	MCR-2	715
MCR-2-R	TCTAGCCCGACAAGCATACC		
CTXM7 -F	GCGTGATACCACTTCACCTC	GR 1-CTXM	265
CTXM8 -R	TGAAGTAAGTGACCAGAATC	CTXM	265
CTXM11 -F	ATCAAGCCTGCCGATCTGGTTA	GR9CTXM	293
CTXM12 -R	GTAAGCTGACGCAACGTCTGC	CTXM	293

CTXM17	TGATACCACCACGCCGCTC	GR2CTXM	341
CTXM18	TATTGCATCAGAAACCGTGGG	CTXM	341
CTXM19-F	CAATCTGACGTTGGGCAATG	GR8 - CTXM	292
CTXM20-R	ATAACCGTCGGTGACAATT	CTXM	292
TEM-F	GCGGAACCCCTATTTG	TEM	963
TEM-R	ACCAATGCTTAATCAGTGAG	TEM	963
SHV-F	TTATCTCCCTGTTAGCCACC	SHV	797
SHV-R	GATTTGCTGATTTCGCTCGG	SHV	797

DNA fragments were analysed by electrophoresis in 2% agarose gel. Eventually, the PCR products were purified and analysed by Sanger sequencer as per the manufacturer's instructions (ABI 3730XL Applied biosystems).

Statistical analysis was done by Medcalc software for calculating Odds ratio, significance level and 95% confidence interval.

RESULTS

A total of 3090 blood culture sets were received during the study period. 820 (26.5%) were two sets and 2270 (73.4%) were single sets. Two hundred and nine patients had bacteraemia of which the first thirty-five patients with multidrug resistant bacteraemia were analysed for probable risk factors and compared with 35 patients with non MDR bacteraemia.

Mean age of patients with MDR bacteraemia was 45.6±16.2 years (median-49 years) and Non MDR bacteraemia is 44.3±17.2years. There were slightly more males in MDR group (53.6% vs 44.8%) compared to Non MDR group.

Table 2: Demographic And Clinical Characteristics Of Patients With Gram Negative Blood Stream Infections

Patient characteristics/risk factors (no.)	MDR (35)	Non MDR (35)	p value	OR(95% CI)
Male sex (41)	22	19	0.46	1.42(0.54-3.70)
Age >60 years (15)	9	6	0.38	1.67(0.52-5.34)
History of malignancy(8)	8	0	0.03*	21.94(1.21-396.99)
Prolonged hospital stay >2weeks (31)	19	12	0.09	2.27(0.86-5.96)
Secondary BSI (18)	15	3	0.0027*	8.00(2.05-31.16)
Patient location in ICU (at time of bacteraemia) (16)	16	0	0.0051	60.07(3.41-1056.69)
Mechanical ventilation (27)	18	9	0.02*	3.05(1.11-8.37)
Escherichia coli (30)	14	16	0.62	0.79(0.30-2.04)
Klebsiellapneumoniae (14)	13	1	0.0064*	18.6(2.2-152.6)
Acinetobacterbaumanii (6)	5	1	0.12	5.66(0.6263-51.26)
Pseudomonas aeruginosa (3)	3	0	0.29	0.45(0.10-1.97)

83.3% of patients with secondary bloodstream infection had MDR bacteraemia (p=0.0027) and Urinary tract was the commonest source of infection in both the groups. Malignancy was significantly associated with MDR bacteraemia(p=0.03) as shown in **Table 2**

All the patients admitted in intensive care units harboured MDR bacteraemia whereas 35% (19/54) of Non-ICU patients had MDR bacteraemia (p=0.0051). 51.4% of MDR bacteraemia patients were on ventilator while 25.7% of Non MDR patients were on ventilator (p=0.02). 57.8% of community onset BSI's were associated with MDR pathogens.

Among the organisms isolated from blood stream infections, Klebsiella was significantly associated with MDR bacteraemia (p=0.0064) as shown in **Figure 1**

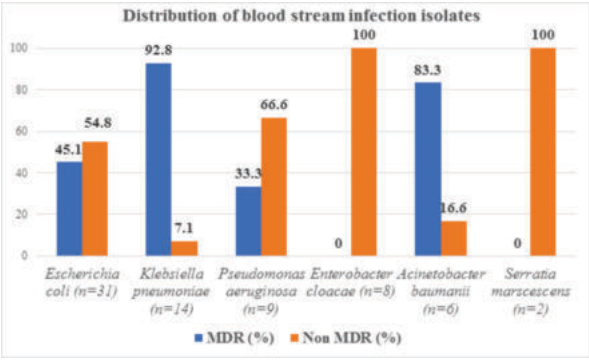

Figure 1: Distribution of MDR & non MDR pathogens causing blood stream infections.

In the MDR group, all *Acinetobacterbaumanii* isolates were sensitive only to Colistin and all pan drug resistant isolates were *Klebsiellapneumoniae* as shown in **Table 3.**

Table 3: Categories Of Resistance Among Gram Negative Pathogens

Organism (n)	MDR(n)	XDR(n)	PDR(n)
Escherichia coli (14)	10	4	0
Klebsiellapneumoniae (13)	3	7	3
Acinetobacterbaumanii (5)	0	5	0
Pseudomonas aeruginosa (3)	0	3	0

bla_{NDM} and/or bla_{OXA-48} were detected in 24 patients; bla_{KPC}, bla_{OXA-23},bla_{IMP},bla_{VIM}, bla_{GES} were not detected in any of the isolates. Two ESBL's- CTX-M7 and CTX-M19 were detected in 7 patients in the MDR group. TEM and SHV were not detected in the present study. bla_{NDM}, bla_{OXA-48} and CTX-M7 ESBL were seen in one patient with pan drug resistance. No enzyme could be detected in three patients; 2 with ESBL phenotype and another with cabapenamase phenotype identified in Vitek panel.All the detected enzymes were observed among four organisms- *Escherichia coli*, *Klebsiellapneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacterbaumanii.*

Both bla_{NDM} and bla_{OXA-48} were detected in 15 patients; Only bla_{NDM} was detected in 3 patients and only bla_{OXA-48} in 6 patients as depicted in **Figure 2**. Among ESBL's, both CTX-7 and CTX-19 were detected in 3 patients, only CTX-7 in 3 patients and only CTX-19 in 1 patient.

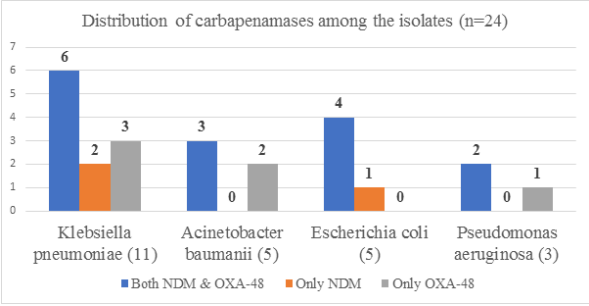

Figure 2: Distribution of carbapenamases among the gram-negative isolates

Of the 19 NDM producers, one *Klebsiellapneumoniae* isolate was pan drug resistant; 9/19 were sensitive only to colistin; 9/19 were sensitive to either cotrimoxazole and or aminoglycosides in addition to colistin. ESBL were identified in only *Escherichia coli* isolates with the exception of one pan drug resistant *Klebsiellapneumoniae* isolate with NDM, OXA-48 carbapenamases in addition to CTX-M7 ESBL. 6/8 ESBL *Escherichia coli* isolates were community associated infections and were resistant to fluoroquinolones. 5/8(62.5%) ESBL isolates were treated with Carbapenems.

Pan drug resistance was observed in 3 patients with *Klebsiellapneumoniae* infection; NDM, OXA-48 along with CTX-M7 was detected in one patient and NDM with OXA-48 was seen in 2nd patient. None of the tested enzymes were detected in the third patient. Two patients were known cases of Acute myeloid leukaemia and the third patient had septic abortion with ARDS. All the three patients were on carbapenems, antifungals and colistin before developing colistin

resistance. Mechanism of colistin resistance could not be found in these three patients.

Mortality was significantly higher in MDR group (40%) compared to Non MDR group (11%) (p=0.0125). 2/3 pan drug/colistin resistant BSI patients died.

DISCUSSION

Blood stream infections can lead to serious life-threatening organ dysfunctions like sepsis and septic shock. This problem is compounded by the multi drug resistant gram-negative organisms with carbapenem and colistin resistance thus limiting the treatment options significantly. Several studies on multidrug resistance and associated risk factors had been conducted since the introduction of carbapenems in 1980's. [12-14]

Significant risk factors for MDR bacteraemia in the present study were infection with *Klebsiellapneumoniae*, secondary BSI, mechanical ventilation, malignancy and treatment in an intensive care unit. Gender and age did not influence the MDR phenotype in the present study. A recent study from Brazil in 2019 observed that male sex, age ≥ 60, previous antimicrobial use, liver disease and bacteraemia caused by K. pneumoniae were associated with MDR bacteraemia. [14]

In the present study, *K. pneumoniae* has been significantly associated with MDR bacteraemia as was found in many studies in Asia, Europe, and South America. [15-18]

ESBL-producing *Escherichia coli*, particularly strains producing CTX-M ESBLs, have been increasingly reported around the world. All ESBL's were detected in Escherichia coli isolates except one pan drug resistant *Klebsiella*isolate and belonged to CTX-M 7 and CTX-M 19 belong to Group 2 and Group 3 respectively. [19] 7/8 ESBL's were detected in community onset infections. This changing epidemiological trend from nosocomial TEM, SHV types of ESBL to community onset associated CTX-M types has been reported in various studies. [20-23]

A multicentric study in India involving four centers in 2019 found that 50% of ESBL E.coli isolates harboured TEM followed by OXA-1 (43%), CTXM-1 (21%), SHV(6%) and CTXM-2 (1%). [24]

Isolates with CTX-M enzymes are resistant to other classes of antimicrobials particularly fluoroquinolones and in present study 87% (7/8) ESBL's were resistant to fluoroquinolones. [7,25,26]

Data from a large clinical trial recommended carbapenem as first-line treatment of ESBL infections outside the urinary tract; in present study 5/8 ESBL patients were treated with carbapenems. [27]

In India, metallo β-lactamases (MBL) are frequently found in the following medically important organisms; *Escherichia coli*, *Klebsiellaspp*, *Pseudomonas aeruginosa* and *Acinetobacter spp*. In the present study, all carbapenamases were observed among *Escherichia coli*, *Klebsiellaspp*, *Pseudomonas aeruginosa* and *Acinetobacterspp* only similar to a recent observation from South India (28). Other organisms like *Serratia*spps and *Enterobacter*spps were negatively associated with MDR phenotype as was found by Leal HF et al 2019. [14]

Treatment options for MBL producing organisms are limited to polymyxins and Tigecycline based therapies. Stability of Aztreonam against MBL's in combination with avibactam to counter ESBL's or Class C beta lactamases which are usually coproduced in MBL organisms is emerging as an effective treatment option in carbapenem resistant organisms. The exception for this combination is seen in infections with *Pseudomonas aeruginosa* and *Acinetobacterspps* where efflux resistance mechanism makes Aztreonam ineffective.

Presence of MBLs tend to confer resistance to most of the beta-lactam antibiotics, including the carbapenems. Furthermore, isolates often tend to be resistant to fluoroquinolones and aminoglycosides, limiting therapeutic options to agents with high rates of toxicity and variable pharmacokinetics , such as colistin (29,30). In the present study nineteen patients had NDM metallo-beta-lactamases in which 13/19 (68.4%) were resistant to aminoglycosides and all 19 of them were resistant to fluoroquinolones. 5 of 19 NDM MBL producers were sensitive to cotrimoxazole, and one isolate was PDR (colistin resistant).

Colistin is the last resort antibiotic for treating carbapenemresistant organisms but colistin resistance is being reported from many centres. A multicentre study from Italy reported that during a 4.5 y period, the colistin resistance increased >3-fold in participating centres and related with a mortality rate of 51%. [31]

24 Colistin resistant Klebsiellapneumoniae were reported from Eastern India including 10 blood culture isolates with 25% mortality. [32] In present study 2 of the 3 patients with colistin resistant BSI died.

Mechanism of colistin resistance could not be evaluated from the targets evaluated-mcr-1 and mcr-2 in the present study. Colistin resistance is mostly due to structural modifications in the bacterial lipopolysaccharide and is caused mostly by mutations in the *mgr* B gene. Appearance of plasmid mediated colistin resistance among Enterobacterales is concerning due to potential for widespread dissemination. In a study from Egypt in 2019, colistin resistance mechanism could be revealed in only 3 of 40 isolates, in present study also mechanism of resistance could not be found in three colistin resistant isolates. [8]

Other targets causing lipopolysaccharide modifications or alternative resistance mechanisms like accumulation of capsular polysaccharide or efflux pumps can contribute to colistin resistance. [33]

CONCLUSION

Treatment options for ESBL and cabapenamase producing organisms are limited and emerging colistin resistance poses a serious challenge in combating life threatening blood stream infections.

Risk factors for MDR bacteraemia in the present study were infection with *Klebsiellapneumoniae*, secondary BSI, mechanical ventilation, malignancy and treatment in an intensive care unit.

Effective infection control measures with judicial use of antibiotics are need of the hour to manage the menace of antimicrobial resistance as newer antibiotics or combination of the existing antibiotics would be unproductive if the preventive measures are not strictly adhered to.

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