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Bacteriological Profile of Septicaemia and Extended Spectrum Beta Lactamases Production in Multi Drug Resistant Strains

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A B S T R A C T

Blood streams infections are important cause of sever morbidity and mortality of patients. The study aimed to find out aerobic bacteriological profile and evaluate emergence of ESBL among multi drug resistant strains. 571 bacterial isolates were subjected to antibiotic susceptibility test for common antibiotics used to treat septicaemia and double disc and Amp C disc test for detection of ESBL and Amp C β lactamases among multidrug resistant strains. In our study maximum patients were from paediatric ward, female patients were more than male but difference is insignificant. There were *Pseudomonas* (19%), *Staphylococcus aureus* (12.9%), *Klebsiella* (12.1%), *E. coli* (11%), *CONS* (11%), *Enterobacter* (8.8%), *Citrobacter* (8.2%), *Acinetobacter* (7%), *Candida* (4%), *Enterococcus* (2.4%), and *salmonella typhi* (1.9%). Gram positive cocci were highly sensitive to vancomycin and teicoplanin and Gram negative bacilli were highly sensitive to Imepenem and quinilones. 21.1% of Enterobacteriaceae produced ESBL, 6.4% produced Amp C β lactamase and 6.3% produced both type of β lactamases. Two isolates of pseudomonas were MBL positive. 23% organism were multi drug resistant (MDR) in all isolates. There was no PDR found in our study. In gram positive cocci 53% were *MRSA* and 43% were *MRCoNS*. Significant difference found between antibiotic susceptibility of ESBL and non ESBL strain. Data of our study provided much needed information on prevalence of antimicrobial resistance amongst pathogens causing blood stream infections in Mewat region. Multidrug resistant organism can result in treatment failure. Therefore it is recommended that any ESBL producing organism according to national committee for clinical laboratory standards (NCCLS) can be reported as resistant to all extended spectrum β lactam antibiotics regardless of susceptibility test results.

Introduction

Blood stream infections (BSIs) are important cause of severe morbidity of patients and ranges from self-limiting infections to life threatening sepsis that require rapid and aggressive antimicrobial treatment. Wasihum *et al* (2015) BSIs cause by wide spectrum of organisms and this spectrum is subject to geographical alteration. These infections may be poly-microbial or mono-microbial. Common organism isolated were *Staphylococcus aureus, Coagulase negative staphylococci,*

Escherichia coli, Pseudomonas spp, Klebsiella spp. Salmonella typhi, Acinetobacter spp, Enterobacter spp. Citrobacter spp, Enterococcus spp, Streptococcus spp, Micrococci and Candida. Usha *et al* (2007)

Various type of β (beta) lactamases increasing worldwide like extended spectrum beta lactamases (ESBL), Amp C β lactamases and mettalo-beta lactamases (MBL). Amutha *et al* (2014) Presence of these enzymes in single isolates reduces the effectiveness of antibiotic chemotherapy. There appears to be a paucity of surveys related to antibiotic resistance from developing countries like Indian subcontinent particularly in this Mewat region.

The main aim of this study was to know common aerobic bacteriological agent of septicaemia and their antibiotic resistance pattern, and emergence of ESBL, Amp C and MBL among multidrug resistant strains.

Duration of study

A prospective study data was conducted from January 2015 to December 2015 at Research Laboratory of Microbiology Department of the SHKM GMC Mewat, Haryana.

Inclusion criteria

The study included all clinical isolates obtained from growth of blood culture came to bacteriology lab both OPD as well as IPD, highly suspected of septicaemia.

Exclusion criteria

1. Patients of HIV and other immunological disorder
2. Patients of diabetes.
3. Patient took antibiotic in last 7days

Methodology

5 ml blood was collected from each adult patient with strict aseptic precaution, and inoculated immediately into 50 ml BHI broth with 0.025% of sodium polyanethol sulphonate as anticoagulant (HImedia). In children (below 12 year) 1 - 2 ml of blood was taken and inoculated in 5 - 10 ml of BHI broth. The broths were sub cultured on 5% blood agar and Mac Conkey agar and chocolate agar after overnight incubation. Subculture was repeated at regular interval up to 7 days until the final result was negative. Positive growth was identified by Gram staining, colony characteristics, and standard biochemical tests. Bailey and scott's *et al* (2007). Antibiotic susceptibility test was put for various antibiotics disc (Hi-Media). Ampicillin (10 µg), Cefuroxime (30 µg), Ceftriaxone (30 µg), Ciprofloxacin (5µg), Amoxicillin-Clavulenate (10/20 µg), Co-trimoxazole (1.25/23.75 µg), Ceftazidime (70 µg), Ceftriaxone (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Amikacin (30 µg), Netilmicin (30 µg), Tetracycline (30 µg), Piperacillin-tazobactam (100/10 µg) and Imipenem (10 µg) (Hi-media, Mumbai). The reference strains used as control for disc diffusion testing were *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212.

The isolate was considered as multidrug resistant (MDR) when non-susceptible to at least one agent in more than three antimicrobial categories/groups and extensively drug resistant (XDR) if non-susceptible to at least one agent in all but two or fewer antimicrobial categories/ groups i.e. bacterial isolates remain susceptible to only one or two categories. Isolate non-susceptible to all agents in all antimicrobial categories was considered as pan drug-resistant (PDR). Magioracos *et al* 2012

ESBL Screening

All the isolates showing resistance to 3rd generation cephalosporins, namely Ceftazidime, Ceftriaxone and Cefotaxime, were further tested for confirmation of β-lactamase production by phenotypic methods. CLSI (2014)

Double Disk Synergy Test

A disc of augmentin (20 μg amoxycillin and 10 μg clavulanic acid) and a 30 μg disc of ceftazidime was placed 15 mm out from edge of Augmentine disc at 90^0 angle so that its inner edge is 15 mm from it. Same was performed with Cefotaxime 30 μg, Ceftriaxone 30 μg, Aztreonam 30 μg, Cefpodoxim 10 μg so that they were spaced 90^0 apart lawn culture of the resistant isolate under test on Mueller-Hinton Agar The zone size around the test antibiotic disc increased towards the Augmentin disc An "enhancement" or extension of the zone of inhibition is seen between any of the cephalosporin antibiotics and the clavulanate containing disks, This phenomenon is often referred to as the "KEYHOLE" effect, or "CLAVULANIC" effect. Gupta et al (2007)

Phenotypic Confirmatory Disk Diffusion Test (PCDDT) for ESBL Detection

Ceftazidime (30 mcg) was used alone as well as in combination with Clavulanic acid (10mcg). Both the disks were placed on MH Agar plates pre-swabbed with the respective culture and incubated at 37 °C for 24 h. An increase in the zone diameter for Ceftazidime-Clavulanic acid by ≥ 5mm was considered positive for ESBL production. Jarlier *et al* (1988)

AmpC Disc Test to Detect Amp C beta Lactamases Production

A lawn culture of *Escherichia coli* 25922 was prepared on MHA plate. Sterile disk of 6 mm were moistened with sterile saline (20 µl) and inoculated with several colonies of test organism. The inoculated disk was then placed beside a cefoxitin disk almost touching on the inoculated plate. The plates were incubated at 37^o c overnight. A positive test appeared as flattening or indentation of the Cefoxitin inhibition zone. Black *et al* (2005)

MRSA and MRCoNS Detection

Detection of Methicillin resistance among *Staphylococcus aureus* isolates was done using 1µg oxacillin disc on Mueller Hinton agar supplemented with an additional 5% NaCl and Cefoxitin disc (30 ug) diffusion test and results interpreted according to CLSI guidelines. Broekema *et al* (2009)

Detection of MBL

MBL production was demonstrated by two methods namely combined disc test with EDTA and Ceftazidime (30µg) – EDTA Double disc synergy test. Marchiaro *et al* (2005)

Result and Discussion

Out of 2681 cases studied 2292 (85.4%) were indoor patients while 389 (14.5%) were from various outpatient departments. Maximum blood samples (59.7 %) were received from Paediatric ward from patients of septicaemia and 11.9 % from Paediatric OPD with PUO, 6.46% from surgery ward from patients of wound infection and PUO and rest were from various other departments. 1403(52.3%) were females and 1278 (47.7%) were males. Female were more than male but difference is not significant (p value < 0.05) Age of patients was ranged from neonates to elderly (0 to 86 years). Maximum patients were from neonatal ICU, mean age of patients were

15.1years. Growth was obtained in 571 samples out of 2681 (21.56 %) in which 96% showed bacterial growth and *Candida spp* was isolated from 4% of the samples. In most of samples there was mono-microbial growth only in 3.25% of cases two or more microorganisms were isolated. In our study 21.1% of Enterobacteriaceae produced ESBL, 6.4% produced Amp C β lactamase and 6.3% produced both type of β - lactamases. Two isolates of pseudomonas were MBL positive. 23% organism were multi drug resistant (MDR) in all isolates. There was no PDR found in our study. In gram positive cocci 53% were MRSA and 43% were MRCoNS.

The results of our study demonstrate the distribution of microbial isolates causing septicaemia and their susceptibility pattern to most commonly used oral and parenteral antimicrobial agents.

In most cases of septicaemia, a single microorganism was isolated from blood, while in 3.25% of cases two or more microorganisms were isolated. The poly-microbial blood stream infections have been reported by various workers with an incidence ranging from 4.7 - 18.7%, most of which were hospital acquired. Garg *et al* (2007), Usha *et al* (2007), Kalpesh *et al* (2014). In our study blood culture positivity rate was 21.29 % that was similar to other study held in India by Garg *et al* (2007)(20.1%) however it was ranged from 8% to 10% by other investigators. Maximum patients were from paediatric age group from new born to 14 years. Manjula *et al* (2005), Vanitha *et al* (2012), Sumita *et al* (2014)

Table No. 1 shows maximum isolates were *pseudomonas spp* followed by *klebsiellae spp* and *staphylococci aureus*. These organisms can survive in the environment for a relatively long time and widely distributed in the hospital environment and have high risk for being transmitted from the environment to the patients through practices that breach infection control measures. Anantham *et al* (2005). This emphasises the need for the establishment of effective and functional infection control programmes in hospitals. Dechen et al (2009). Antibiotics used for susceptibility testing for Gram-negative isolates, Ciprofloxacin, Cefotaxime, Gentamycin and Amoxycillin / Clavulanate was good effective against *Enterobacteriaceae*, whereas for non-fermenters like *Pseudomonas spp.* and *Acinetobacter spp.* Ofloxacin and Amikacin was more active. However, the combination of Piperacillin-tazobactam and Imipenem put up for all Gram-negative isolates showed the highest activity among all antibiotics used for these isolates. Our findings were similar to other investigators. Usha et al (2007) Vinod et al (2011) (Table No. 3) In gram positive cocci maximum isolates were *S. aureus* which was found to be less susceptible to Ampicillin, Erythromycin, and Clindamycin. Other antibiotics like Cotrimoxazol, Ciprofloxacin, Gentamycin, Amoxycillin/Clavulenate have good activity against all gram positive *cocci* and none of the strains showed resistance to Vancomycin and Teicoplanin. (Table No. 2) They could be used in multidrug resistant strains. Similar results have been reported by other workers. Our findings were similar to other investigators. Manjula *et al* (2005), Usha *et al* (2007), Garg *et al* (2007)

In our study MRSA were 53% and MRCoNS were 43%. All gram positive *cocci* including MRSA, Enterococcus spp. and Coagulase negative *Staphylococci* were 100% sensitive to Vancomycin. Our findings are similar to Karthikeyan *et al* (2001), who reported that 66% of *Staphylococcus aureus* isolated from cases of neonatal sepsis were methicillin resistant.

Table.1 Distribution of Microorganisms Isolated from Blood Cultures

Organism	No. of isolates	Percentage
Pseudomonas	108	19%
Staphylococcus aureus	74	12.9%
Klebsiella	70	12.1%
E coli	63	11%
CONS	63	11%
Enterobacter	50	8.8%
Citrobacter	47	8.2%
Acinetobacter	40	7%
Candida	23	4%
Enterococcus	12	2.4%
Salmonella	11	1.9%
Micrococci	10	1.7%
Total	571	

Table.2 Antibiotic Sensitivity Patterns of Gram-Positive Organisms in Percentage

Antibiotics	*CoNS (n=63)*	*Staphylococcus aureus (n=74)*	*Enterococci (n= 12)*
Vancomycin	63(100%)	74(100%)	12(100%)
Erythromycin	36(57.1%)	38(51.3%)	5(41.6%)
Clindamycin	39(61.9%)	43(58.1%)	6(50%
Cefotaxime	43(68.2%)	50(67.5%)	-
Cefepime	43(68.2%)	58(78.3%)	-
Ciprofloxacin	54(85.74%)	64(86.46%)	12(100%)
Teicoplanin	59(93.6%)	70(94.5%)	12(100%)
Gentamycin	56(89%)	65(87.8%)	10(83.3%)
Amoxycillin-clavulenate	48(76.1%)	58(78.3%)	9(75%)
Ampicillin	31(49.2%)	29(39.1%)	1(8%)

Table.3 Antibiotic Sensitivity Patterns of Gram-Negative Organisms

	Psedomonas n=108	*Citrobacter n=47*	*Klebsiella n=70*	*Acinetobacter n=40*	*E.coli n=63*	*Enterobacter n=50*	*Salmonella n=11*
Ampicillin	-	13(27.6%)	48(68.5%)	27(67.5%)	41(65%)	25(50%)	7(63.6%)
Amikacin	98(90.7%)	43(91.4%)	58(82.8%)	36(90%)	56(88.8%)	42(84%)	11(100%)
Ceftazidime	56(51.8%)	26(55.3%)	42(60%)	22(55%)	43(68.2%)	30(60%)	7(63.6%)
Cefazolin	-	31(65.9%)	37(52.8%)	23(57.5%)	34(53.9%)	32(64%)	6(54.54%)
Cefepime	-	34(72.2%)	37(52.8%)	-	33(52.3%)	30(60%)	5(45.45%)
Ciprofloxacin	-	41(87.2%)	58(82.8%)	33(82.5%)	54(85.7%)	44(88%)	11(100%)
Piperacillin-tazobactam	98(90.7%)	40(85.1%)	62(88.5%)	35(87.5%)	55(87.3%)	45(90%)	7(63.6%)
Imepenem	95(87.9%)	42(89.3%)	56(80%)	37(92.5%)	61(96.8%)	50(100%)	10(90.9%)
Gentamycin	95(87.9%)	41(87.2%)	64(91.4%)	31(77.5%)	58(92%)	41(82%)	9(81.8%)
Cefotaxime	-	34(72.2%)	49(70%)	31(77.5%)	55(87.3%)	35(70%)	6(54.54%)
Netilmycin	97(89.8%)	36(76.5%)	54(77.1%)	32(80%)	57(90.4%)	41(82%)	8(72.72%)
Amoxycillin-clavulenate	-	38(80.8%)	51(72.8%)	36(90%)	52(82.5%)	42(84%)	10(90.9%)
Aztreonam	-	26(55.3%)	31(44.2%)	23(57.5%)	33(52.3%)	34(68%)	2(18.1%)
Cotrimoxazol	-	34(72.2%)	51(72.8%)	35(87.5%)	54(85.7%)	40(80%)	7(63.6%)
Ofloxacin	89(82.4%)	-	-	-	-	-	-
Tetracyclin	-	26(55.3%)	54(77.1%)	33(82.5%)	43(68.2%)	35(70%)	2(18.1%)

Table.4 Comparison of Various Studies from Various Places

Study	Place	ESBL
Baby padmini et al 2004	Tamilnadu	41%
singhal et al 2005	Gurgaon	64%
Sehgal et al 2007	New delhi	61.3%
Shridhar Rao et al 2007	Karnatak	62.9%
Sinha et al 2008	Jaipur	64.8%
Dechen et al 2009	Sikkim	34%
Chatergee 2010	Chandigarh	53.8%
Akujobi 2010	Nigeria	57.6%
Nasrin et al 2010	Iran	28.6%
Sanjay et al 2010	Mysore	23.1%
K Aruna et al 2012	South Mumbai	34.7%
Amutha et al 2014	Chennai	67.3%
Present study 2016	Haryana	21.1%

Table.5 Antibiotic Susceptibility Pattern of Esbl and Non Esbl Isolates

Antibiotic	ESBL n=120	Non ESBL n=451	P value
Ampicillin	0	275(61%)	<0.05
Cotrimoxazol	42(35%)	311(69%)	<0.05
Tetracycline	57(47.5%)	302(67%)	<0.05
Ciprofloxacin	64(53.4%)	383(85%)	<0.05
Gentamycin	78(65%)	415(92%)	<0.05
Imepenem	113(94%)	451(100%)	<0.05
Piperacillin+Tazobactam	79(65.8%)	392(87%)	<0.05
Amoxycillin clavulenate	53(44%)	329(73%)	<0.05

Isolation of MRSA was 31.25% as per Gandhi *et al* (2013), and it was 33% according to Patel *et al* (2014). So screening for MRSA in every *Staphylococcus aureus* isolated will be of immense value for providing efficient patient care.

In our study 23% isolates were multi drug resistant (MDR). The infection caused by MDR organisms is more likely to prolong the hospital stay, increase the risk of death, and require treatment with more expensive antibiotics. Gene for ESBL, Amp C and MBL are often carried by plasmids, facilitating rapid spread between micro-organism. Jain *et al* (2003) Presence of these enzymes in single isolates reduces the effectiveness of β lactam and β lactam inhibitor combinations, while Amp C and MBL confer resistance to Carbepenems also. Often these enzymes are co-expressed in the same isolates. In almost all cases, antimicrobial therapy is initiated empirically before the results of blood culture are available. Keeping in mind the high mortality and morbidity associated with septicaemia, right choice of empiric therapy is of important. MRSA and MRCoNS indicated hospital acquired infection that needed strict infection control measures. The irrational use of broad spectrum β-lactam antibiotics has led to a marked increase in the incidence of ESBL in Gram negative organisms. ESBL were detected in 21.1% of Enterobacteriaceae. This is different from other studies from North India in which 61.5% were ESBL producers. Sehgal *et al*

(2007). 6.4% isolates showed Amp-C beta lactamases in those isolates. This is similar to observation made by Singhal *et al* (2005) which showed Amp-C beta lactamases in 8% isolates. The difference in percentage of isolation of ESBL compared to few other studies could be due to regional variations. Distinct regional variations have been detected in the incidence of ESBL producing isolates. Restricted use of Cephalosporins significantly reduced the incidence of ESBL. A significant difference (P value < 0.05) found between antibiotic susceptibility of ESBL and non ESBL strains. ESBL producing strains were more resistant to antimicrobial agents including Ampicillin, Cotrimoxazol, Tetracycline, Ciprofloxacin and Gentamycin. Imipenem was found to be most effective antibiotic against ESBL producing strain while in non ESBL strains Imipenem resistance was zero. ESBL producing strains were resistant to more antimicrobial agents than non ESBL producing strains. The highest rate of resistance in ESBL negative strains were seen against Ampicillin which was significantly lower than ESBL producing strains. None of the first line antibiotics used singly showed high susceptibility to all the Gram-negative bacilli, so a combination of two or more drugs is recommended to cover the broad range of possible pathogens which may be difficult to distinguish clinically. This may prevent the emergence of resistance as they may have additive or synergistic antimicrobial activity.

Simple hygienic measures, such as hand washing practices, the use of sterile equipment, cohorting (i.e. grouping patients with similar infections in the same location) of patients and attending staff for MRSA and ESBL can help prevent the further spread of these resistant strains. This study stresses that antimicrobial resistance is a global problem and emphasizes the need for surveillance and promotion of correct and restrictive antibiotic policies including specific antibiotic therapy after studying sensitivity pattern. This will halt the further spread of MRSA and ESBL and improve the prognosis in cases of sepsis.

In conclusion, these data provided much needed information on the prevalence of antimicrobial resistance amongst pathogens causing blood stream infections our area. The rise in antibiotic resistance in blood isolates emphasises the importance of sound hospital infection control, rational prescribing policies, and the need for new antimicrobial drugs and vaccines. Resistance to broad spectrum β lactams (ESBL, AmpC and MBL) can result in treatment failure. Therefore it is recommended that any ESBL producing organism according to national committee for clinical laboratory standards (NCCLS) can be reported as resistant to all extended spectrum β lactam antibiotics regardless of susceptibility test results. Our results seem helpful in providing Useful guidelines for choosing an effective antibiotic in cases of septicaemia and for choosing salvage therapy against hospital resistant strain.

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