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Common Isolates among Suspected Cases of Septicemia with a Special Emphasis on Multidrug Resistant Strains

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A B S T R A C T

To isolate common organisms from suspected cases of septicemia with their antimicrobial susceptibility pattern and know the prevalence of MDR strains by detecting MRSA isolates, ESBL, AmpC and Carbapenemase production. Blood samples were collected from suspected cases of septicemia and incubated in BacT/ALERT 3D. Subculture was done on Blood agar, nutrient Agar, MacConkey Agar and Chocolate Agar and identification was done by standard biochemical reactions. AST was done by Kirby Bauer Disc Diffusion method with MRSA, ESBL, AmpC and Carbapenemase producing isolates detected by using Cefoxitin, Ceftazidime and Ceftazidime + Clavulanic acid, Cefoxitin and Ceftriaxone and Modified Hodge Test respectively. Out of 300 samples, 24.66% were culture positive with Gram Negative Organisms being predominant [Klebsiella pneumoniae most common (29.72%) followed by *E. coli*] with highest resistance to Cefixime (91.67%), Cefoperazone + Sulbactam (83.33%). Coagulase Negative Staphylococcus (5.40%) was most common Gram Positive organism isolated which showed highest resistance to Ampicillin and Cefixime (75%). 18 were MDR strains with 16.21% ESBL producers, 6.71% Carbapenemase producers, 2.7% MRSA isolates. No AmpC producers were detected. Gram Negative Septicemia was more predominant with highest resistance to cefixime and sensitivity to Polymyxin B and Imipenem. 24.32% of total isolates were MDR strains. Continued Surveillance studies are needed for implementation of control measures to limit spread of MDR strains.

Introduction

Bacteremia is the presence of bacteria in the blood. Septicemia is a condition in which bacteria multiply within the blood and their toxic products cause harm to the host producing an infection. It is the leading cause of death with mortality rates ranging from 20% to 50%, especially in Intensive Care Units, prolonging the hospital stay of the patients. Microbial invasion of blood stream can have serious immediate consequences including shock, multiple organ failure, DIC and death (Patricia M. Tille; Stephane Hugonnet *et al.*, 2004).

ICU patients create an environment for infection because of debilitating effect of

prolonged hospitalization as well as usage of various medical equipments like airways and catheters (Mohanasoundaram, 2011). A critical issue is the time one can declare the blood culture as positive or negative (Vinod Kumar, 2005). Even if the blood cultures are done, the report of isolation and susceptibility is available after 72hours or more with the use of Ampicillin and an aminoglycoside or a 3rd generation cephalosporin being the empirical treatment (Bhattacharjee *et al.,* 2008).

Surveillance to describe varied pathogens causing sepsis and their changing antimicrobial susceptibility pattern is important (Kaistha *et al.,* 2010).

With the production of ESBLs, Carbapenemases in case of Gram Negative Bacteria and MRSA emerging as major threats, this situation raises a serious concern regarding faster microbial recovery using automated BacT/ALERT 3D blood culture system and appropriate antibiotic use.

In the light of the above challenges, present study was aimed to isolate the microbial agents from suspected cases of septicemia along with antibiotic sensitivity pattern with special emphasis on MRSA, ESBL, AmpC and Carbapenemase producing strains.

Aims and objectives

To isolate the common organisms from the blood samples of suspected cases of septicemia from the hospitalized patients in PICU, NICU and Intensive Care Unit

To study the antimicrobial susceptibility pattern of the various bacterial isolated.

To know the prevalence of Multidrug Resistant strains among the various organisms isolated by detecting MRSA, ESBL, AmpC and Carbapenemase production.

Materials and Methods

Ethical clearance

Blood Samples were obtained from patients following approval of the study by the Institutional Ethical Committee.

Study design

It was a prospective study conducted in Department of Microbiology, Narayana Medical College, from July 2014 to June 2015 over a period of 1 year with a sample size of 300

Inclusion criteria: Suspected cases of septicemia and Pyrexia of unknown origin

Exclusion criteria: Patients on antibiotics

Method of blood collection

Two blood samples were collected in from different sites from the suspected cases of sepsis by following the standard aseptic precautions at bed side

Volume of blood from adult: 6-10ml

Volume of blood from neonate: 1 – 2ml

Volume of blood from children: 3 - 5ml

The blood was collected in Blood culture bottles (Adult and Pediatric bottles) and was kept in BacT/ALERT machine for incubation.

Once the growth was there, machine beeped (6-24hrs).

Gram-stain

Smears were made from the positive samples, heat-fixed and stained by Gram-stain and the preliminary report was given to the clinician.

Culture and identification

Plating was done on Blood Agar, Nutrient Agar, MacConkey's Agar, and Chocolate Agar and incubated at 37^0C aerobically for 18-24hours. Growth was identified by standard biochemical reactions (Oxidase test, Catalase Test, Coagulase test, Indole test, Citrate Utilization Test, TSI, Urease Production Test, MR, VP and Hugh Leifson's Oxidative Fermentative test) Depending on the morphology of colonies, the presumptive identification of the organism was made. Candida species were identified by Germ Tube Test.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was done by the Kirby-Bauer disc diffusion method. The antibiotics used were according to CLSI guidelines using HiMedia discs.

The following drug discs were used for Enterobacteriaceae: Ampicillin (10µg), Cefixime (5µg), Cotrimoxazole (25µg), Ofloxacin (5µg), Amikacin (30µg), Polymyxin B (50 units), Amoxycillin + Clavulanic acid (30µg), Cefoperazone + Sulbactam (75/30µg), Piperacillin + Tazobactam (100/10 µg), Imipenem (10µg), Aztreonam (30µg)

The following discs were used for Non-Fermenters: Ceftazidime (30µg), Cefepime (30µg), Cotrimoxazole (25µg), Ciprofloxacin (5µg), Gentamicin (10µg), Amikacin (30µg), Polymyxin B (50 units), Cefoperazone + Sulbactam (75/30µg), Piperacillin + Tazobactam (100/10µg), Meropenem (10µg), Aztreonam (30µg)

The following discs were used for Gram Positive Bacteria: Ampicillin (10µg), Oxacillin (1µg), Cefixime (5µg), Azithromycin (15µg), Ofloxacin (5µg), Amikacin (30µg), Vancomycin (30µg), Clindamycin (2µg), Amoxycillin + Clavulanic acid (30µg), Piperacillin + Tazobactam (100/10µg), Linezolid (30µg)

Drug resistance pattern detection

Testing for MRSA

The test strains resistant to Oxacillin were tested for Methicillin resistance using cefoxitin discs**.** Lawn culture of the test strain was made onto Mueller-Hinton Agar (MHA) and Cefoxitin disc (30µg) was placed in the centre. The zone of inhibition was measured after overnight incubation at 37^0C.

Interpretation: ≤ 21mm = MRSA, ≥ 22mm = MSSA

Testing for ESBL production

Lawn culture of the test strain was made onto Mueller Hinton Agar and Ceftazidime and Ceftazidime+ Clavulanic acid discs were placed and incubated aerobically overnight at 37^0C.

Interpretation: A ≥ 5mm increase in zone diameter for Ceftazidime versus zone diameter for Ceftazidime + Clavulanic acid was considered as ESBL producer.

Testing for Carbapenemase production

1:10 dilution of the 0.5 McFarland suspension of E.coli ATCC 25922 was taken and lawn culture was done onto Mueller Hinton Agar. Ertapenem disc was placed; the test organism was streaked in a straight line from the edge of the disc and incubated overnight at 37^0C. Interpretation: Enhanced growth at the intersection of the streak and zone of inhibition was considered positive for carbapenemase production. No enhanced growth at the intersection was considered negative for carbapenemase production

Testing for AmpC production

Lawn culture of the test strain was made onto Mueller Hinton Agar and Cefoxitin and Ceftriaxone discs were placed with a distance of 20mm from each other and incubated aerobically overnight at 37^0C.

Interpretation: A flattening or blunting of the zone between the cefoxitin disc and the ceftriaxone disc was considered as AmpC β-lactamase producers.

Results and Discussion

Among 300 clinically suspected cases of septicemia selected for the study, blood culture positivity rate was 24.66%. Studies conducted by Joshi *et al.,* (2000) (25%), Muley *et al.,* (2015) (26.6%), and Iregbu *et al.,* (2006) (22%) showed similar rates on contrary to higher rates of isolation by Parikh Madhubala and Singh Nandon (1995) (47%), Surya Kirani and Sailaja (2015) (42%) and Jaslyn *et al.,* (2013) (42%). The variation in culture positivity can be attributed to the volume of blood collected, type of blood culture medium and automated blood culture systems. Male preponderance was observed (78.37%) similar to observation made by Anitha Sharma *et al.,* (1993) (74%) in their study.

Of the culture positives, Gram negative organisms were the commonest isolates (83.78%), similar to Gram Negative preponderance in Culture positive cases reported by Sharma Anitha *et al.,* (1991) (85%), Mahapatra *et al.,* (2002) (88.45%) in contrast to Gram Positive isolates as most common reported by Kingsley *et al.,* (2013) (62.6%) and Starakis *et al.,* (2010) (58.5%). Low *Candida* isolation rates (2.43%) were found to be similar to findings by Shashi Gandhi *et al.,* (2013) (2.6%) and Desai *et al.,* (2011) (3.57%).

Among the Gram Negative organisms, most common organism isolated was *Klebsiella pneumoniae* (33.33%) followed by *Escherichia coli* (16.66%), *Citrobacter* koseri (16.66%) and *Acinetobacter baumannii* (16.66%). Studies done by Surya Kirani and Sailaja (2015) and Rahul Kamble and Rajesh Ovhal (2015) reported similar pattern of isolation with *Klebsiella pneumoniae* (33.5%) being most common followed by *Escherichia coli* (14.3%), *Acinetobacter* species (9.5%) and *Klebsiella pneumoniae* (35.55%) respectively. Coagulase Negative *Staphylococcus* (CONS) was most common Gram Positive organism isolated (8.1%) similar to findings by Rahul Kamble and Rajesh Ovhal (2015) (16.9%), Jaslyn *et al.,* (2013) (13.92%) and Mahapatra *et al.,* (2002) (2.3%). Leon *et al.,* mentioned that presence of CONS in the blood can no longer be considered as contaminant especially in patients in critical care units where it is associated with significant mortality.

Out of 12 culture positive pediatric patients, 100% had lethargy, 83.33% presented with respiratory distress, tachycardia and chest retractions and 33.33% with hyperthermia, in comparison to reports wherein majority of sepsis cases presented with refusal to feeds (42.7%), fever (41.7%) by Shrestha *et al.,* and lethargy (73.24%) by Rahul Kamble and Rajesh Ovhal (2015). In case of adult patients, hyperthermia, tachycardia and tachypnoea were most common parameters observed (100%) keeping in mind report by Coburn *et al.,* (2012) that fever alone cannot be a criteria to predict bacteremia (Table 1–15 and Fig. 1).

Most common predisposing factors evaluated were found to be respiratory condition (48.38%) followed by renal condition (32.25%), DM (22.58%) similar to study conducted by Starakis *et al.,* (2010) who found lower respiratory tract infection (39.1%) as major risk factor followed by UTI (25.7%).

Table.1 Distribution of total samples

	Number	%
Adult	222	74
Pediatric	78 (NICU – 46; PICU – 32)	26
Total	300	100

Table.2 Number of culture positive samples

	Number	%
Culture positives	74	24.66
No growth	226	75.33
Total	300	100

Table.3 Gender distribution among culture positive samples

Gender	Number	%
Male	58	78.37
Female	16	21.62
Total	74	100

Table.4 Distribution of culture positive samples

	Number	%
Adult	62	83.78
Pediatric	12	16.21
Total	74	100

Table.5 Organism wise distribution

ORGANISM	NUMBER (N=74)	%
GRAM NEGATIVE ORGANISMS	**62**	**83.78**
Klebsiella pneumoniae	22	29.72
Escherichia coli	12	16.21
Pseudomonas aeruginosa	10	13.51
***Citrobacter* koseri**	8	10.81
Acinetobacter baumannii	6	8.10
***Citrobacter* freundii**	2	2.70
***Klebsiella* oxytoca**	2	2.70
GRAM POSITIVE ORGANISMS	**6**	**8.10**
Coagulase Negative Staphylococci	4	5.40
Staphylococcus aureus	2	2.70
FUNGAL	**6**	**8.10**
Candida albicans	6	8.10

Table.6 Organisms isolated from culture positive adult patients

ORGANISM	NUMBER (N=62)	%
GNB		
Klebsiella pneumoniae	18	29.03
Escherichia coli	10	16.12
Pseudomonas aeruginosa	10	16.12
***Citrobacter* koseri**	6	9.67
Acinetobacter baumannii	4	6.45
***Citrobacter* freundii**	2	3.22
***Klebsiella* oxytoca**	2	3.22
GPC		
Coagulase Negative Staphylococci	2	3.12
Staphylococcus aureus	2	3.12
FUNGAL		
Candida	6	9.67

Table.7 Organisms isolated from culture positive pediatric patients

ORGANISM	NUMBER (N=12)	%
GNB		
Klebsiella pneumoniae	4	33.33
Escherichia coli	2	16.66
***Citrobacter* koseri**	2	16.66
Acinetobacter baumannii	2	16.66
GPC		
Coagulase Negative Staphylococci	2	16.66

Table.8 Screening criteria in culture positive adult patients

CRITERIA	NUMBER (N=62)	%
Hyperthermia	62	100
Tachycardia	62	100
Tachypnoea	62	100
Leukocytosis	60	96.77
Leukopenia	2	3.22

Table.9 Screening criteria for culture positive pediatric patients

CRITERIA	NUMBER (N=12)	%
Lethargy	12	100
Respiratory distress	10	83.33
Tachycardia	10	83.33
Chest retraction	10	83.33
Poor activity	6	50
Hyperthermia	4	33.33
Poor feeding	2	16.66

Table.10 Predisposing factors among culture positive adult patients

PREDISPOSING FACTOR	NUMBER (N=62)	%
Respiratory Condition	30	48.38%
Renal condition	20	32.25
Diabetes Mellitus	14	22.58
Hypertension	12	19.35
>65yrs	12	19.35
Cardiovascular condition	10	16.12
Gastrointestinal condition	8	12.90
Surgery	6	9.67
Pancreatitis	6	9.67
Trauma	4	6.45
Poisoning & Snake bite	4	6.45

Table.11 Predisposing factors among culture positive pediatric patients

Predisposing factor	Number (n=12)	%
Preterm	6	50
Respiratory infections	4	33.33
CNS infections	4	33.33
Low birth weight	2	16.66

Table.12 Antibiogram of Gram positive cocci

S.No	Antibiotic	Organism							
		CONS (n=4)				*Staphylococcus aureus* (n=2)			
		S	S%	R	R%	S	S%	R	R%
1	Ampicillin	1	25	3	75	0	0	2	100
2	Oxacillin	2	50	2	50	1	50	1	50
3	Cefixime	1	25	3	75	1	50	1	50
4	Azithromycin	2	50	2	50	1	50	1	50
5	Ofloxacin	3	75	1	25	1	50	1	50
6	Amikacin	3	75	1	25	1	50	1	50
7	Vancomycin	4	100	0	0	1	50	1	50
8	Clindamycin	4	100	0	0	1	50	1	50
9	Amoxycillin + Clavulanic acid	4	100	0	0	2	100	0	0
10	Piperacillin + Tazobactam	4	100	0	0	2	100	0	0
11	Linezolid	4	100	0	0	2	100	0	0

Table.13 Antibiogram of *Pseudomonas aeruginosa*

S.No	Antibiotic	Organism (n=10)			
		S	S%	R	R%
1	Ceftazidime	10	100%	0	0
2	Cefipime	8	80%	2	20%
3	Cotrimoxazole	8	80%	2	20%
4	Ciprofloxacin	10	100%	0	0
5	Gentamicin	8	80%	2	20%
6	Amikacin	8	80%	2	20%
7	Polymyxin B	2	20%	8	80%
8	Cefoperazone + Sulbactam	2	20%	8	80%
9	Piperacillin + Tazobactam	10	100%	0	0
10	Meropenem	10	100%	0	0
11	Aztreonam	2	20%	8	80%

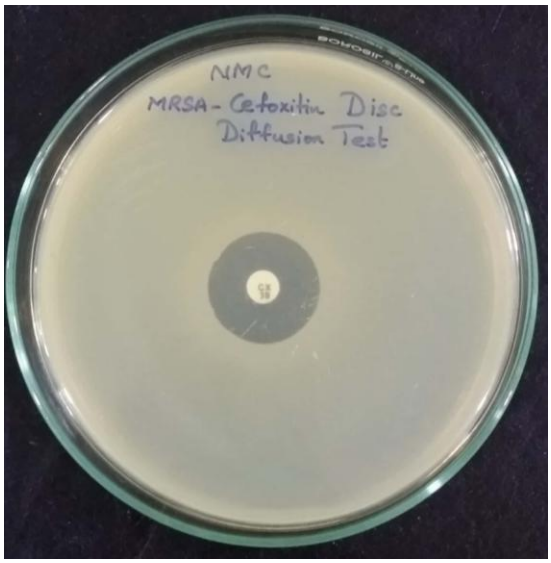
Table.14 Antibiogram (Resistance pattern) of Gram negative bacteria

S.No.	Antibiotic	Organism			
		Klebsiella **(n=24)**	***E. coli*** **(n=12)**	***Citrobacter*** **sp (n=10)**	***A. baumannii*** **(n=6)**
		R (%)	**R (%)**	**R (%)**	**R (%)**
1	Ampicillin	20 (83.33)	10 (83.33)	10 (100)	6 (1000
2	Cefixime	22 (91.67)	8 (66.67)	10 (100)	2 (33.33)
3	Cotrimoxazole	16 (66.67)	8 (66.67)	6 (60)	4 (66.67)
4	Ofloxacin	10 (41.66)	6 (50)	0 (0)	0 (0)
5	Amikacin	6 (25)	2 (16.67)	2 (20)	0 (0)
6	Polymyxin B	0 (0)	0 (0)	0 (0)	0 (0)
7	Amoxycillin + Clavulanic Acid	14 (58.33)	2 (16.67)	8 (80)	2 (33.33)
8	Cefoperazone + sulbactam	20 (83.33)	10 (83.33)	10 (100)	6 (1000
9	Piperacillin + Tazobactam	6 (25)	2 (16.67)	8 (80)	0 (0)
10	Imipenem	3 (12.5)	1 (8.33)	2 (20)	0 (0)
11	Aztreonam	18 (75)	12 (100)	8 (80)	4 (66.67)

Table.15 Multidrug resistant strains among culture positive patients

	Number	%
MDR strain	18	24.32
MRSA	2	2.7
ESBL producer	12	16.21
AmpC producer	0	0
Carbapenemase producer	5	6.7

Fig.1 A: MRSA detection: Cefoxitin Disc Diffusion Test; B: ESBL detection: Combination Disc Diffusion Test



A

B

Yinnon *et al.,* (1996) reported UTI (58%) as major risk factor. In case of pediatric patients, 50% were preterm, 33.33% has underlying respiratory, CNS infections and Low Birth Weight as major risk factors. Studies by Surya Kirani and Sailaja (2015) and (Vinay *et al.,* 2009) also reported LBW (61.6%, 70%) and preterm (51.6%, 68.4%) as major risk factors.

CONS isolated in our study showed highest resistance to Ampicillin and Cefixime (75%), 50% sensitivity to Oxacillin and Azithromycin. *Staphylococcus aureus* showed 100% resistance to Ampicillin and 50% resistance to Oxacillin, Cefixime, Azithromycin, Ofloxacin, and Amikacin. Similar resistance patterns were observed by Vinod Kumar (2005) [Penicillin (67.8%), Cloxacillin (55.6%)] and Kingsley *et al.,* (2013) [Ampicillin (57.1%)]. 100% sensitivity was observed by all Gram Positive organisms to Amoxycillin+ clavulanic acid, Piperacillin + Tazobactam and Linezolid. *Klebsiella* species were found highly resistant to Cefixime (91.67%), Cefoperazone + Sulbactam, Ampicillin (83.33%) with highest sensitivity to Polymyxin B. Carbapenem resistance in Gram Negative Organisms was found to be 9.6% with similar rates reported by Roy *et al.,* (2002) (10%) and Saghir *et al.,* (13%). Rahul Kamble and Rajesh Ovhal (2015) observed highest resistance to Ampicillin (100%) and 3rd Generation Cephalosporins (70%) and 100% sensitivity to Imipenem. In case of *Pseudomonas aeruginosa*, highest resistance was found against Polymyxin B, Cefoperazone + Sulbactam, Aztreonam (80%) with 100% sensitivity to Ceftazidime, Ciprofloxacin, Piperacillin + Tazobactam and Meropenem.

A study by Roy *et al.,* (2002) showed high resistance to Cefotaxime (50%) and low resistance to Ciprofloxacin, Amikacin (10%) in concordance with our study though Shashi Gandhi *et al.,* (2013) reported 33.33% and 16.67% resistance to Ciprofloxacin and meropenem respectively. Such high frequency of resistance observed towards β lactam antibiotics by various organisms might be due to indiscriminate use of them as 1st line drugs.

Out of 74 culture positive isolates, 18 are Multidrug Resistant Strains. 16.21% of the isolates were ESBL producers with highest being *Klebsiella* species followed by *E. coli* and *Citrobacter*. Studies done by Iregbu *et al.,* showed 11.4% ESBL producers similar to our study though higher rates were reported by Saghir *et al.,* (2009) and Rahul Kamble and Rajesh Ovhal (2015) 58% and 82.5% respectively with *Klebsiella* most common ESBL producer. 6.7% isolates were Carbapenemase producers with *Klebsiella* being predominant organism. Similar low rates were reported by Castanheira *et al.,* (2006-07) (2.7%) and Roy *et al.,* (2002) (10%). In contrary, Gupta *et al.,* (2006) reported 22% Carbapenemase producers. Low MRSA rates of 2.5% reported by us were correlated with study conducted by Neelam Kaistha *et al.*, (2010) (11.11%), though higher rates were reported by Raza *et al.,* (75% in CONS and 41.66% in *S. aureus*).

Though the mortality rate of septicemia has gone down from 90% prior to antibiotic era to 24-25 % after the antibiotics came into use, the production of ESBLs, Carbapenemases and evolution of MRSA have made therapeutic options limited. Unfortunately ampicillin no longer seems to have significant activity against most of the organism, showing no role in empirical treatment any more. Ciprofloxacin, Amikacin and Imipenem turned out to be better alternatives in case of Gram Negative Septicemia producing economical relief to the patients. ICUs being the place where antibiotic usage is highest, patient to patient transmission of resistant organisms might be an important factor for Multi drug resistance emergence.

With such varied isolation rates, it is important to conduct surveillance studies for implementation of control measures thereby limiting the spread of Multidrug Resistant isolates for better patient outcome.

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