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BACTERIAL AGENTS AND ANTIMICROBIAL SUSCEPTIBILITY AMONG CHILDREN WITH INFECTION IN BLOOD STREAM.

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ABSTRACT

Background: According to studies, BSI are the infection present in blood , through which it can circulate to each and every organ in the body. Studies have also shown that their presence can lead to various risk factors specially when we talk about neonatal septicemia. **Aim:** To evaluate & assess the bacterial presence and their AMSP among children below 14. **Material & method:** A prospective study with 1000 patients blood samples collection were done for evaluation of presence of bacteria witth the help of BC in different agar plates after incubation followed by assessing the bacteria sensitivity and resistance against medicine. **Result:** We found that out of 1000 blood samples 315 showed +ve BC with mainly males being affected approx 175 (55.55%) and finally high prevalence of gram +ve than –ve . **Conclusion** : Careful evaluation should be done before instituting therapy to avoid unnecessary use of antibiotics.

Keywords: BC, prevalence, antibiotics, bacteria, BSI, risk factor,medicine, gram +ve & -ve , blood sample and sensitivity

INTRODUCTION

According to a study bloodstream infections, commonly known as BSIs. They serve as an indicator of the presence of bacteria in the circulation. They are regarded as one of the most hazardous situations that may occur in relation to infectious diseases due to the fact that they pose a risk to each and every organ in the body.[1] As one of the most frequent causes of morbidity and mortality in neonates and children, blood stream infections are a prevalent occurrence in the pediatric age range. According to estimates, around twenty to fifty percent of children living in countries with low levels of economic development are impacted by bloodstream infections. Furthermore, it is thought that one out of every five infants is affected by these disorders.[2] The most common bacteria that cause bacteremia *include Staphylococci, Streptococci, Enterococci, Escherichia, Klebsiella, Pseudomonas, Enterobacter, Haemophilus, and Neisseriagenera*.[3] According to a study, they are one of the most important cause of morbidity & mortality throughout the world.[3] Thus, this an challenging problem that can varyingly impact on world economy & social living. Children suffering from septicemia exhibit symptoms such as fever, respiratory distress, rapid heart rate, fatigue, aversion to eating, or extreme tiredness. Potentially severe consequences such as shock, multiple organ failure, and disseminated intravascular coagulation may arise. Therefore, bloodstream infections are considered to be one of the most critical conditions, making it crucial to promptly detect and identify the pathogens present in the bloodstream. While clinical signs and symptoms are useful in diagnosing septicemia, the primary method of diagnosis is bacteriological culture.[2] Therefore , according to a research, presence of bacteria in blood circulation is a threat to every oragn of the body.[4] Moreover, according to a study, blood culture(BC) has the most important way to detect there presence for definitive diagnosis followed by treatment.[5,6]

Thus, our present study was designed and implemented to determine the bacterial etiology of blood stream infections in pediatric patients and to examine antibiotic susceptibility patterns of the isolated organisms.

AIM

To evaluate & assess the bacterial agents and their antimicrobial susceptibility pattern(AMSP) among children below 14 years of age having BSI.

MATERIAL & METHOD

For our prospective type of study , we had obtained ethical clearance from our institutional ethical committe and informed consent form filled by all the included 1000 patients , who have reported to the department of Microbiology Bacteriology Laboratory at GMERS Medical college, Sola , Ahmedabad starting from October ,2020 ending to October 2022 .

INCLUSION CRITERIA

1. All the samples recieved in the ward
2. Below 14 year of age

EXCLUSION CRITERIA

1. Patient more than 14 year of age
2. Those who denied the consent for participation.

LABORATORY METHOD

BC was done by manual by BHI broth prepared in-house & automated methods were ready to use Bactec BC bottles. They were further processed for organism identification & anti-microbial sensitivity. BC samples were collected by aspectic precautions in pediatric BC bottle and sample was collected from cubital vein around 2-5 ml of blood. In addition to this, skin site was sisinfected with povidine-iodine or spirit to avoid contamination. The transport of sample to bottle was done at room temperature.

SAMPLE PROCESSING

a. Manual

Day 1) BC bottle recieve in lab & placed in incubator for 24 hrrs at 37˚C.

Day 2) Look for signs of growth = turbidity, hemolysis, gas bubbles and surface pellicle formation.

Those bottles who did not show any sign of growth were incubated for around 5 days more and checked for any sign of growth.

b. Automated Machine

Day 1) Bactec BC bottle recieve in lab and were placed . Program was set for total of 5 days in between whenever the sample was positive, it was indicated by machine.Thereafter, the bottle was removed from machine.

PROCESSING OF POSITIVE BC

All the positive BC were subcultured on Nutrient agar, 5% sheep blood agar and Mac Conkey agar by proper aseptic precautions.

EXAMINATION OF CULTURE PLATE

1. Lactose fermenter/ non-lactose fermentor
2. Size, shape, surface, margin, texture, colony count
3. Hemolytic property
4. Swarming property

GRAM STAINING

It is routinely done for all the colonies to determine positive or negative. Its interpretation is as follows:-

SHAPE	COLOUR	ARRANGEMENT	INFERENCE
Spherical	Violet	Clusters, pairs and chains,tetrads	Gram positive cocci
Rod	Pink		Gram negative bacilli
Oval budding cells	Violet		Yeast cells

MORPHOLOGY OF POSITIVE STAIN

1. Nutrient Agar Plate:
    a) 1-2mm, Small, circular, opaque, low-convex colonies
    b) Tiny colonies were also seen
2. Blood Agar Plate :
    a) Hemolytic/ npon- hemolytic with opaque colonies
    b) Tiny non- hemolytic pin- point colonies
    c) Creamy colonies with uneven margin
3. MacConkey Agar Plate:
    a) Lactose fermenting small opaque colonies
    b) Tiny magenta pin colonies

IDENTIFICATION OF POSITIVE ORGANISM

Along with gram staining test, biochemical test was also done which were as follows:-

1. Catalase test (slide / tube test)
2. Coagulase test (Slide / tube test)
3. Urease
4. Bile esculinage (enterococcus species)

AMS TESTING :

After identification of causative organism the management of BSI includes early & appropriate treatment by antimicrobial therapy. This test was done by Kirby-Bauer Disc diffusion method. Medium used for susceptibility test was Muller-Hinton Agar. A single colony of organism was tested & inoculated into peptone water & it should be matched with 0.5 McFarland turbidity standard.

Interpretation was as follows:-

Zone of inhibition of bacterial growth around antibiotic disc using clinical & laboratory standard institute , USA guideline:-

a. Staphylococcus aureus – ATCC 25923
b. Escherichia coli- ATCC 25922
c. Pseudomonas aeruginosa – ATCC 27853

RESULT

BLOOD CULTURE	NO. OF PATIENTS (n = 1000)	%
POSITIVE	315	31.5
NEGATIVE	685	68.5

TABLE 1: BC

In our study, table 1 showed that BC was seen positive for 315 patients (31.5%) .

AGE	TOTAL	%
0-28 DAYS	142	45.07%

29 DAYS – 5 YEARS	114	36.19%
6-14 YEARS	59	18.73%
TOTAL	315	100%

TABLE 2: AGE DISTRIBUTION

In our study, table 2 showed that age group 0- 28 days showed most BC positive 142 (45.07%) followed by 29 days to 5 years with 114 and 6- 14 year of age with 59 patients in number respectively.

GENDER	NUMBER OF PATIENTS	%
MALE	175	55.55
FEMALE	140	44.45
TOTAL	315	100

TABLE 3: GENDER

In our study, table 3 showed that males were in majority for positive results i.e. 175 (55.55%) followed by females with 140(44.45%) repectively.

ORGANISM TYPE	TOTAL	%
GRAM POSITIVE ORGANISMS	191	60.63
GRAM NEGATIVE ORGANISMS	124	39.37

TABLE 4: ORGANISM TYPE

In our study, table 4 showed that positive were in majority with 191 (60.63%) followed by negative with 124 (39.37%) respectively.

DISTRIBUTION OF SPECIES	NUMBER	%
Coagulase negative Staphylococcus	160	83.73
Staphylococcus aureus	18	9.42
Streptococcus species	4	2.09
Enterococcus species	9	4.71
TOTAL	**191**	**100**

TABLE 5: GRAM POSITIVE SPECIES

In our study, table 5 showed that coagulase negative staphyloccci were the most prominant isolates with 160 ((83.73%) followed by staphylococcus aureus with 18 (9.42%) , then enterococcus species with 9 (4.71%) and finally , streptococcus species with 4(2.09%) respectively.

DISTRIBUTION OF ISOLATES	NUMBER	%
Klebsiella species	43	34.67
Escherichia coli	18	14.51
Pseudomonas species	13	10.48
Salmonella typhi	16	12.9
Enterobacter species	2	1.61
Acinetobacter species	32	25.08
TOTAL	**124**	**100**

TABLE 6: GRAM NEGATIVE

In our study , table 6 showed that , klebsiella species showed most prominant isolate with 43(34.67%) followed by acinetobacter species with 32(25.08%) , then E.coli with 18 (14.51%) , S. Typhi with 16 (12.9%) , pseudomonas species with 13 (10.48%) and enterobacter species with 2 (1.61%) respectively.

Distribution of isolates	**NUMBER**	**%**
MRCONS	119	66.85%
MRSA	11	6.17%

TABLE 7: ISOLATE FOR STAPHYLOCOCCUS

In our study, table 7, 119 MRCONS (66.85%) showed staphylococcus out of 178 .

Antibiotic tested	**Drug conc.(mcg)**	**MRSA (11)**	**MSSA (7)**	**MRCONS (119)**	**MSCONS (41)**	**Streptococc- us species (4)**	**Enterococc-us species (9)**
Penicillin G	10 U	11(100%)	6(85.7 %)	117(90.7%)	14(34 %)	0(0%)	4(44.5 %)
Ciprofloxacin	5	9 (81.1 %)	6(85.7 %)	85(71.4%)	85(71 .4%)	_	6(66.7%)
Cotrimoxazole	25	5(45.4 %)	1(14.3%)	79(66.3%)	9(22 %)	_	_
Erythromycin	15	8(72.1 %)	3(42.8 %)	81(69.7%)	5(12.9 %)	0(0%)	5(55.5 %)
Clindamycin	2	4(36.3%)	1(14.3%)	57(47.9%)	5(12.9%)	0(0%)	_
Tetracycline	30	3(27.2%)	0(0 %)	31(26 %)	2(4.8 %)	0(0%)	2(22.3%)
Linezolid	30	0(0%)	0(0 %)	4(3.36 %)	0(0%)	0(0%)	0(0%)
Cefoxitin	30	11(100 %)	0(0 %)	119(100%)	0(0%)	_	_
Gentamicin	10 U	7(63.6 %)	1(14.3%)	51(42.8%)	0(0%)	_	_
Chloramphenicol	30	1(9 %)	0(0 %)	25(21 %)	2(4.8 %)	_	_
Vancomycin (MIC)	30	0(0%)	0(0 %)	1(0.84 %)	0(0%)	0(0%)	3(33.3%)
Ampicillin	10	_	_	_	_	0(0%)	4(44.5%)
Ceftriaxone	30	_	_	_	_	0(0%)	_

Teicoplanin	30	_	_	_	_	0(0%)	2(22.3 %)
High level Gentamicin	120	_	_	_	_	0(0%)	4(44. 5%)

TABLE 8: ANTIBIOTIC RESISTANT (Gram +ve)

In our study, table 8 showed antibiotic resistant pattern of MRSA, MSSA , MRCONS, MSCONS and stretptococci. Methicillin resistance staphylococcus aureus & CONS showed high degree of resistance to other classes of antibiotics as well as Erythromycin, Clindamycin, Ciprofloxacin, Cotrimoxazole and Gentamicin. MSCONS &MSSA somehow showed resistance to other antimicrobials like Erythromycin, Clindamycin, Ciprofloxacin, Cotrimoxazole and Gentamicin. Majority of CONS & all staphylococcus aureus were sensitive to Vancomycin, Linezolid and Chloramphenicol. Among GPC streptococcus are highly susceptible to Erythromycin,Clindamycin,Ceftriaxone.Thus, Enterococcus was highly sussceptible to Linezolid, Tetracyclin, Vancomycin, Ampicillin, Ceftriaxone.

TOTAL ISOLATES (KLEBSIELLA AND E.COLI	**ESBL PRODUCERS**	**%**
63	16	25.39 %

TABLE 9: ESBL for Gram –ve

According to table 9, ESBL producers in our study was 25.39%.

Antibiotic tested	**Drug conc.(mcg)**	**Entero bacter specie s (2)**	**Esche richia coli (18)**	**Klebs iella speci es (43)**	**Pseudo monas species (13)**	**Acinet obacter species (32)**	**Salm onella typhi (16)**
Ampicilin	10	_	14(7 7.7%)	_	_	_	1(6.2 5%)
Ampicillin/S albactum	10 / 10	_	13(7 2.2%)	_	_	_	_
Piperacillin/ Tazobactam	100/1 0	1(50 %)	10(5 5.5%)	23(5 3.4%)	_	18(56 %)	_
Ceftriaxone	30	2(100 %)	13(72 .2%)	35(81 %)	_	_	1(6.2 5%)
Cefotaxime	30	2(100 %)	13(72 .2%)	36(8 3.7%)	_	_	_
Cefotaxime/ Clavulanic acid	30 10	1(50 %)	8(44. 4%)	19(4 4%)	_	_	_
Ceftazidime	30	2(100 %)	13(72 .2 %)	34(79 %)	3(23%)	26(81. 20 %)	_

Ceftazidime/ Clavulanic acid	30 10	0(0%)	5(27. 7%)	17(39 .5 %)	_	_	_
Cefipime	30	2(100 %)	11(61 .1 %)	33(76 .7 %)	3(23 %)	23(71. 8%)	_
Ciprofloxaci n	5	2(100 %)	13(72 .2 %)	30(6 9.7%)	2(15.3 %)	_	16(10 0 %)
Levofloxacin	5	1(50 %)	12(6 6.6%)	25(5 8%)	3(23 %)	23(71. 8%)	3(18. 75%)
Gentamycin	10	2(100 %)	8(44. 4%)	32(74 .4 %)	3(23 %)	20(62. 2%)	_
Amikacin	30	1(50 %)	9(50 %)	29(67 .4 %)	1(7.7 %)	15(46. 85%)	_
Cotrimoxazo le	25	2(100 %)	5(27. 7 %)	18(4 1.8%)	_	19(59. 4%)	2(12. 5 %)
Tetracycline	30	2(100 %)	4(22. 2%)	7(16. 2 %)	_	8(25%)	_
Chloramphe nicol	30	2(100 %)	1(5.5 %)	11(2 5.5%)	_	_	1(6.2 5 %)

TABLE 10: ANTIBIOTIC RESISTANT (Gram -ve)

In our study, table 10 shows that among GNB most common isolated organism is Klebsiella species followed by AcinetobacterSpecies.All gram negative isolates are sensitive to Colistin. most GNB are highly resistance to Ampicillin, Ceftriaxone, Cefotaxime, intermediate resistance to Tetracycline , Levofloxacin and Gentamycin. Low level resistance to Imipenem and Chloramphhenicol. Thus, S. Typhi was highly resistant to Ciprofloxacin & Levofloxacin while sensitive for Ampicillin, Ceftriaxone & Tetracycline.

DISCUSSION

In our study we have collected 1000 blood samples out of which 315 showed positive BC . The results of our study correlate well with the result of the study done by Ajeitha L et al .[7] Culture positivity rate in our study was highest in the age group of 0-28 days followed by 29 days - 5 years.This was comparable with the previous study of Abebaw A et al.[3] In our study, 175 (55.55%) were male patients which were in majority followed by 140 (44.45%) patiets were females . Similar results were seen in the study done by Habyarimana et al [8] Furthermore, frequency of isolation of gram positive organism were higher uptoo 60.63% than gram negative upto (39.37%) . Simlar results were seen in Dash M et al study also [9]

Out of gram +ve organism in our study, CoNS followed by S. aueus was seen more. These results coinsides witth the results of study done by Kirchoff et al [10] Dash M et al.,[9] Abebaw A et al., [3]. Moreover, the isolates were reported as true pathogens of BSI in 59 patients out of 160 patients (36.87%) in our study. The study in Basel of Elzi Let al., [11] andin Aligarh of Khan Fet al.,[12] also reported 64.52% and 65.21% blood culture contaminants respectively. In our study, we also found that majority of gram+ve showed resistance to Penicillin,

erythromycin followed by clindamycin. The same was seen in the study of Kirchoff et al [10] While in our study, all gram+ve organisms are sensitive to Linezolid and Vancomycin and this is same was also reported in a study done by Muhammad et al.,[13]

CONCLUSION

In our research, the overall prevalence of bacteremia was 31.5%. Both gram +ve & -ve were discovered to contribute to the high incidence of bacterial pathogens in bloodstream infections with majority of the isolates were CoNS. Therefore, The main forces driving the increase in antimicrobial resistant bacteria are the inappropriate use of antibiotics. Specific antibiotic utilization strategies like antibiotic restriction, combination therapy and antibiotic escalation and de-escalation may help to decrease or prevent the emergence of resistance.

The high prevalence of CoNS is mostly attributable to skin contamination. The practice of proper venipuncture and handwashing techniques by medical staff arerecommended to circumvent the difficulty of interpreting BC. Thus, careful evaluation should be done before instituting therapy to avoid unnecessary use of antibiotics. This will definately help us in improving infection control practices by formulating policies for empirical AMT.

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