Original Research Article

Bacterial isolates from the Pediatric patients and its correlation with microbiological flora in tertiary care hospital

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Abstract

Background: Infection is a health hazard of great expense and significance affecting the final outcome of treatment. Hospital indoor air contains a diverse range of microbial population.

Objective: To study microbiological organism and their antibiotic sensitivity isolated from patients' blood culture and to correlate this micro-organism with pattern of microbiological flora in tertiary care hospital.

Materials and methods: All the patients who fulfilled study criteria were subjected for blood culture analysis and prospectively observed for clinical conditions and there outcome. Antimicrobial susceptibility testing was performed for all blood culture isolates. Case fatality study was a comparison between deaths in blood culture positive patients with reference to resistance to first line antibiotics. A comparison was made between the type of organism found in cultures of samples taken from patients and the type of organism obtained from sites near the patients during the same time interval.

Results: out of 3144 sample sent for blood culture, 888 (28.2%) samples came out to be positive. most isolates of klebseilla, Acinobacter, and E. coli were resistant to ampicillin (97 to 98%), gentamycin (70 to 80%) and cephalosporins (65% to 100%). Most of the gram negative isolates were sensitive to levofloxacin and Piperacillin-tazobactam. Staphylococcus aureus were resistant to

ampicillin (90.4%). Staphylococcus aureus were sensitive with vancomycin by 98.6%. Case fatality due to gram –ve organisms were 19.45%. Gram –ve bacteria were dominating pathogens in both prefumigation samples from various sites of ICU and wards and blood culture sample from patients admitted in ICU and wards i.e. 32 (72.7%) and 815 (91.8%) respectively. It sets up a correlation between source of infection and sepsis in patients admitted to ICU and wards.

Conclusion: Most organism (gram positive and gram negative) were resistant to first line antibiotics such as ampicillin, gentamycin, cephalosporins. Antibiotic combination with piperacillintazobactam or levofloxacin had better sensitivity among all gram negative organisms. Gram positive organisms were susceptible to vancomycin. Most deaths occur in patients infected with first line antibiotic resistant strains. Strong association found between source of bacterial infection and culture sample positivity.

Key words

Case fatality, Microbiological flora, Bacterial isolates.

Introduction

Infection is a health hazard of great expense and significance affecting the final outcome of treatment. Hospital indoor air contains a diverse range of microbial population. Infection control is a quality standard and is essential for the wellbeing and safety of patients, staff and visitors. It affects most departments of the hospital and involves issues of quality, risk management, clinical governance and health and safety.

Good hospital hygiene is an integral component for preventing hospital acquired infections. The microbiological quality of the air is one of the significant parameter for controlling hospital acquired infection. This study will focus on such current aspects.

Materials and methods

This prospective observational study was carried out in a tertiary care centre and included clinically suspected patients of septicaemia admitted from age day 1 to 12 year. Eligibility criteria for 0-1 month was neonates having risk of EOS(Early onset sepsis), preterm babies with weight <1kg or home delivered before starting antibiotics, neonates presenting with respiratory distress or birth asphyxia with difficult resuscitations requiring intensive therapy such as CPAP and ventilation, neonates having risk factors or clinical features of LOS (Late Onset Sepsis) and for age: 1 month- 12 years eligibility criteria was patients having multi organ failure due to sepsis, patients having signs and symptoms suggestive of septicaemia, patients suffering from pyrexia of unknown origin, severe acute malnourished patients, patients in high risk category of septicaemia such as those with damaged valves seen in Rheumatic Heart Disease.

All the patients who fulfilled the study criteria were subjected for blood culture analysis and parents were informed about the study and detailed informed consents were taken and other details and observations were recorded after prior permission of the parents. Study proforma was previously approved by Institutional Ethical committee (IEC). Details such as age of the patient, weight of the patient, sex of the patient diagnosis outcome in form of survival are recorded.

Patients who were enrolled in the study were prospectively observed for clinical conditions and there outcome, their investigations, based on the clinical condition and advised by concerned consultants, were reviewed. Patient's diagnosis and treatment decisions were taken by concerned consultants under whom the patient was being treated. An analysis was conducted on all blood culture reports obtained during March 2014 to March 2015, from above mention class of

patients admitted at tertiary care hospital. Blood culture samples were collected by on duty residents taking care of the patients under treatment. Training for procedure to take blood culture samples was shown to nursing staff and all resident doctors by assistant professor of paediatrics at the beginning of study. For all newborn who were admitted on day 1 of life, their samples were sent after 24 hours while those patients admitted or coming to us after 24 hour of life such as referred patients, their culture samples were taken at the time of admission if they were fulfilling inclusion criteria.

Blood culture sample was collected from a peripheral vein under aseptic conditions. The local site was cleansed with 70% alcohol and povidone iodine (1%) followed by 70% alcohol again. Blood cultures were done in a brain heart infusion biphasic medium. Approximately 2 ml blood was inoculated into the brain heart infusion broth and sent to microbiology department within 30 min to 1hour of collection and incubated at 37 degree centigrade. Blood culture broth that showed no microbial growth within 7 days was reported as culture negative, only after result of routine subculture on blood, Mc Conkey and chocolate agar. Antimicrobial susceptibility testing was performed for all blood culture isolates by Kirby-Bauer disc diffusion method as recommended by National Committee for clinical laboratory standards guideline. In this study no minimum inhibitory concentration (MIC) was given by microbiologists.

The results of antimicrobial sensitivity were reported as sensitive or resistance to any particular drug. Reports of their microorganisms including their antibiotic sensitivity were collected and analysis was done for antibiotic resistance, sex predominance, type of organism isolated, organisms found as per timing of infection.

Data comparison was done in terms of timing of sample collection, presentation of the patient, clinical response of the patient and patient outcome based on organism isolated and its resistance was compared by case fatality rate and survival was studied. For case fatality study we have studied three bacteria that i.e. klebsiella, E. coli, and staphylococcus aureus. Case fatality study was a comparison between the number of deaths in blood culture positive patients with reference to resistance to first line antibiotics. For Klebsiella and E. coli first line antibiotics included were cefotaxim, amikacin, ampicillin and gentamycin. Sensitivity to anyone of this was considered as sensitive blood culture isolate and if bacteria was not sensitive to any of this of four it was included in resistance category. For staphylococcus aureus sensitivity was taken for Vancomycin. Housekeeping samples for ICU of the hospital were taken twice every month. Sample from the ward were sent once every month from various sites as a routine for ICU, basin 1, warmer 2, oxygen probe 1, bed 1, procedure trolley 1. Samples from doctors and paramedical staff hand, nail bed, nares and mobiles were also sent. Consents were taken from doctors and staff for sample collection. Samples were collected in random fashion from doctors and staff who were present on the day of sampling.

Procedure

Samples were also collected from floor, wall, cot, sampling area, humidifiers, oxygen supply units, ventilators, procedure trolley etc. samples were also collected with swab stick by rubbing the stick in circular manner or the desire area of sampling. These swab sticks were then carefully packed and sent for cultures to the microbiology department on the same day of collection to minimize any kind of artefact. These swab stick were incubated and the microbe were isolated from them which were than reported back to the pediatric hospital mentioning the type of organism isolated and the site from which it was isolated. Once an area was sampled for microbes it was fumigated and closed for 24 hours and samples were taken from this area immediately after completion of 24 hours as post fumigation samples in the similar fashion. Post fumigation samples reports were also reported back for if any growth found. A comparison was made

between the type of organism found in cultures of samples taken from patients and the type of organism obtained from sites near the patients during the same time interval.

Results

In present study out of 3144 sample sent for blood culture, 888 (28.2%) samples came out to be positive. It was observed that out of total positive samples 888 (100%), 538 (60.5%) were males and 350 (39.4%) were females. Out of 805 samples from ICU, majority were males 491 cases (61%). Similarly from wards, 47(56.6%) cases were males and 36 (43.4%) were females as per **Table – 1**.

Table - 1: Number of positive samples from patient.

	Positive Samples	**Total sample sent**
ICU	805(32.2%)	2502
WARD	83(9.35%)	642
TOTAL	888(28.2%)	3144

In present study, out of total positive samples from ICU and Wards, 815 (91.8%) cases were positive for gram –ve bacteria and 73 (8.2%) cases were positive for gram gram+ve bacteria. In gram -ve bacterial pool 591 (66.6%) were Klebsiella, 104 (11.7%) were E. coli, 66 (7.4%) were Acinetobacter and 54 (6.1%) were pseudomonas respectively. In present study, out of total positive sample from ICU, 585 (72.7%) cases were categorized under early onset sepsis (<72 hrs) and 220 (27.3%) cases as late onset sepsis as per **Table - 2**.

Most isolates of klebseilla, Acinobacter, and E. coli were resistant to ampicillin (97 to 98%), gentamycin (70 to 80%) and cephalosporins (65% to 100%). Most of the gram negative isolates were sensitive to levofloxacin and Piperacillin-tazobactam. Overall sensitivity (means value) of piperacillintazobactam and levofloxacin to all common gram negative organisms was 70% and 55.2% respectively. Staphylococcus was resistant to ampicillin (90.4%). Staphylococcus was sensitive with vancomycin by 98.6%.

Case fatality due to three most common gram-ve bacterial isolates from blood culture i.e. Klebsiella (19.9%), E. coli (18.9%), Staph aureus (15.3%).Case fatality due to gram –ve organism were 19.45%. In klebsiella 36 (16.6%) deaths out of 217 sensitive microbes to first line antibiotics and 76 (22.1%) deaths out of 345 resistant microbes to first line antibiotics. In E. coli 3 deaths out of 26 sensitive microbes to first line antibiotics and 15 deaths out of 69 resistant microbes to first line antibiotics**.** In staph aureus 1 (14.1%) deaths out of 50 microbes sensitive to vancomycin and 1 (50%) deaths out of 2 microbes resistant to vancomycin.

In present study, out of total samples sent before fumigation (44), 30 (68.2%) were positive from ICU Sites and 14 (31.8%) were from wards. Post fumigation samples were free of any microbial flora. Out of total 44 positive prefumigation samples, 32 (72.7%) were positive for gram –ve bacteria and 12 (27.3%) were positive for gram +ve bacteria. Gram –ve bacteria were dominating pathogens in both prefumigation samples from ICU and wards and culture sample from patients admitted in ICU and wards i.e. 32 (72.7%) and 815 (91.8%) respectively. It sets up a correlation between source of infection and sepsis in patients admitted to ICU and wards. Association between prefumigation positive samples from sites and blood culture isolates is highly significant and thus microbes isolated from sites could be source of infection for patients (**Table – 3**).

Table - 4 signified that floor was the commonest site of microbial isolation and klebsiella being the commonest organism to be isolated in ICU prefumigation samples. Floor was the commonest site of microbial isolation and klebsiella being the commonest organism to be isolated in wards prefumigation samples.

It signifies the increment in positive blood culture from Sept-March and increment in death rate in culture positive patients during the same

period. In E. coli increment in blood culture samples from Sep-March and similar increment in mortality during September-march. While in staph aureus decline in positive blood culture during later half of the year but the mortality increased significantly (**Table – 5**).

Table - 2: Distribution of microbes.

TOTAL POSITIVE SAMPLES						
	GRAM +VE	**GRAM –VE**				TOTAL
	Staph Aureus	**Klebsiella**	**E. Coli**	**Pseudomonas**	**Acinobacter**	
ICU	52(6.5%)	562(69.8%)	95(11.8%)	41(5.1%)	55(6.8%)	805(100%)
WARD	21(25.3%)	29(34.9%)	9(10.8%)	13(15.7%)	11(13.3%)	83(100%)
TOTAL	73(8.2%)	591(66.6%)	104(11.7%)	54(6.1%)	66(7.4%)	888(100%)

Table - 3: Pattern of antimicrobial resistance.

Antibiotics	**Antimicrobial Resistance (%)**				
	StaphAureus	**Klebsiella**	**E. Coli**	**Pseudomonas**	**Acinobacter**
PIPERACILLIN-TAZOBACTUM	86.3	28.3	38.5	20.4	31.8
LEVOFLOXACIN	28.8	32.3	40.4	68.5	37.9
AMIKACIN	100	62.6	64.4	70..4	59
GENTAMICIN	94.5	77	46..3	46.3	74.2
COTRIMOXAZOL	57.6	85.2	-	-	72.7
VANCOMYCIN	1.4	-	-	-	-
CEFOTAXIM	91.8	81.2	65.4	100	78.8
AMPICILLIN	90.4	98	98	53.7	97
MEROPENAM	10	7.5	7.5	9.6	8.6

Discussion

The percentage of positive blood culture was 32.2% in present study. Similarly Dipen Patel, et al. [1] found 23.59% positive blood culture, Arpita, et al. [2] found 31.57%, Chandra Mathur, et al. [3] showed 37.6% culture out of total sample sent. Sarita Kamarth, et al. [4] shows least sensitivity to ampicillin in gram –ve bacteria, klebsiella 14% sensitive, E. coli 18% sensitive, Acinetobacter 17%. Dipen Patel, et al. [1] observed, Most isolates of Klebsiella, Enterobacter, Acinetobacter and E. coli were resistant to ampicillin (96% to 100%), gentamycin (56% to 100%) and cephalosporin (83% to 100%). Chandra Mathur, et al. [3] shows Klebsiella 100% sensitive to meropenam. In present study, Klebsiella is 93.7% sensitive to meropenam. Klebsiella and E. coli are 67.6% and 55.6% resistant to gentamycin as in present study.

Over all case fatality in culture positive samples in present study was 19.45% which was comparable with Dipen, et al. [1] study (15.04%) and Sundaram, et al. [5] study (20%). It also shows that case fatality was highest by klebsiella (19.9%) followed by E. coli (18.9%) and Staph aureus (15.3%) and was comparable with study of Dipen Patel, et al. [1]. It was concluded that majority of deaths among klebsiella positive were among culture positive patients and were due to first line antibiotic resistant strains. Deaths in patients infected with E. coli were mostly due to first line resistant microbial strains. Deaths in

patient infected with staph aureus were mostly in vancomycin sensitive bacterial strains. P value was > 0.05; we conclude that most of these deaths were due to other co-morbid condition. It was found that initially during first six months of study Klebsiella isolates from ICU and wards declined, however the Klebsiella isolates from patients' blood culture was increasing gradually. However during the period of Sept-March when klebsiella isolation from wards and ICU was on a declining mode the blood culture isolates kept on mounting and comparing with mortality (15.6% during first six months compared to 26.5% during next six months). Blood culture isolates follow the isolation pattern of microbes from atmosphere of wards and ICU throw out the year. As it was clearly seen decline in blood culture isolates with decline in E. Coli isolates form environment of wards and ICU. Isolates of Staph Aureus from ICU, wards blood culture followed parallel route throw out the year. It is found that increase in mortality was associated with higher resistant bacteria which were most probably imported from outside. Theoretically it can be said that these bacteria have provided cross resistance to flora of ICU and wards by plasmid transfer which ultimately resulted in higher mortality.

Table - 4: sites of isolation and distribution of microbes.

ICU		
MONTHS	**SITE OF ISOLATION**	**ORAGANISM ISOLATED**
MARCH '14	Floor	**KLEBSIELLA**
APRIL '14	Floor, Bed, Humidifier	**KLEBSIELLA** **E.COLI** **KLEBSIELLA**
MAY '14	Bed, Wall	**KLEBSIELLA** **KLEBSIELLA**
JUNE '14	Floor, Table	**KLEBSIELLA** **STAPH AUREUS**
JULY '14	Floor, Table	**KLEBSIELLA** **E.COLI**
AUG '14	Floor, Table	**KLEBSIELLA** **E.COLI**
SEPT '14	Ventilator, Floor	**E.COLI** **STAPH AUREUS**
OCT '14	Floor	**KLEBSIELLA** **E.COLI**
NOV '14	Table, Wall	**KLEBSIELLA**
DEC '14	Floor, Table, Humidifier	**KLEBSIELLA** **E.COLI** **KLEBSIELLA**
JAN '15	Floor, Oxygen Tube	**STAPH AUREUS** **KLEBSIELLA**
FEB '15	Floor, Bed	**STAPH AUREUS** **STAPH AUREUS**
MARCH '15	-	-

This could be true for Klebsiella which was the major killer. However E. Coli and Staph. Aureus followed the isolation pattern of microbes from wards and ICU were from endogenous flora.

Table - 5: Annual pattern of distribution of Klebsiella and its mortality.

Time interval	Culture positive sample	Deaths
March-August	211	42(19.9%)
Spet-March	262	62(23.7%)

Conclusion

Klebsiella was the most common organism in neonatal sepsis. Staphylococcus was the commonest among the gram positive organism. Most organism (gram positive and gram negative) were resistant to first line antibiotics such as ampicillin, gentamycin, cephalossporins. Antibiotic combination with piperacillintazobactam or levofloxacin had better sensitivity among all gram negative organisms. Gram positive were susceptible to vancomycin. Maximum case fatality was associated with klebsiella. Most deaths occur in patients infected with first line antibiotic resistant strains. Strong association found between source of bacterial infection and culture sample positivity. High prevalence of resistant organisms in this study proves that regular surveillance of organism and their sensitivity pattern is required.

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