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ORIGINAL ARTICLE

Identification of Resistance Pattern in Different Strains of Bacteria causing Septicemia in Human at Lady Reading Hospital of Khyber Pakhtunkhwa

ASIF AZIZ[1], MUHAMMAD ZAHOOR[2], AAMIR AZIZ[2], MUHAMMAD ASGHAR[3], JUNAID AHMAD[4], GOHAR ISLAM[5], NAHEED[6]
[1]*Trainee Registrar Orthopedic Unit, MTI KGN Bannu*
[2]*Institute of Biological Sciences, Sarhad University of Science and Information Technology, Peshawar,Khyber Pakhtunkhwa, Pakistan*
[3]*Department of Pathology KTH/KMC Peshawar*
[4]*Department of Microbiology Hazara University Mansehra,KPK, Pakistan*
[5]*Department of Dermatology MTI HMC/KGMC Peshawar*
[6]*Center of Biotechnology and Microbiology, University of Peshawar, Khyber Pakhtunkhwa, Pakista*
Corresponding author: Naheed, Email: drnaheedhmc@gmail.com

ABSTRACT

Septicemia is one of the most life threatening problems and a major cause of morbidity and mortality particularly in neonates and children. The current research was carried out on identification and antibiotics susceptibility pattern of pathogens responsible for septicemia. A total of 121 samples were collected from septicemia suspected infants admitted in lady Reading Hospital (LRH), Peshawar, Pakistan. Among 121 samples, 43 (35.53%) blood cultures were positive for septicemia. Out of 43 cases, 27 (62.79%) showed growth of gram positive while 16 (37.20%) for Gram negative bacteria. The most common Gram positive isolates were Staphylococcus aureus 19 (70.37%) and Enterococcus spp., 8 (29.62%). Among Gram negative isolates were Klebsiella pneumonia 6 (37.5%), followed by pseudomonas aeruginosa 4 (25%) Escherichia coli 4 (25%), Citrobacter freundii 1 (6.25%) and Serratia 1 (6.25%). Staphylococcus aureus isolates were resistant to ampicillin followed by doxycycline, cefoxitin, gentamycin, but were sensitive to linezolid. Enterococcus spo., were resistant to gentamycin, ampicillin but sensitive to linezolid. Enterococcus spp., were resistant to gentamycin, ampicillin but sensitive to doxycycline followed by teicoplanin and minocycline. The P. aeruginosa were resistant to ampicillin followed by teicoplanin and minocycline. The P. aeruginosa were resistant to ampicillin followed by minocycline, aztreonam but sensitive to cefepime, gentamycin. The K.pneumonia isolates were resistant to ampicillin followed by aztreonam, gentamycin, doxycycline, minocycline but sensitive to cefepime. The E.coli XI isolates were resistant to ampicillin and cefepime followed by aztreonam, doxycycline, minocycline but sensitive to gentamycin. In this research, resistant pattern of 43 isolates were identified out of which 24 (55.81%) were MDR, 12 (27.90%) were sensitive, 7 (16.27%) were XDR and luckily no PDR isolate was found.

Keywords: Septicemia, Sepsis,Antibacterial resistance

INTRODUCTION

Blood Stream infection is one of the most commonly encountered problems in pediatric nurseries and a major cause of morbidity and mortality particularly in developing countries (Bhutta et al.,2003). It encompasses various systemic infections of neonates such as septicemia, pneumonia, meningitis, osteomyelitis, arthritis, and urinary tract infections. Other superficial infections like conjunctivitis and oral thrush are not usually enlisted under neonatal sepsis. Septicemia refers to the presence of bacteria and their toxins in the sterile region (blood stream) of the body with subsequent fever and prostration. It is an important cause of morbidity and mortality among newborn babies, particularly in preterm infants having low weight right after delivery (Adams et al.,2008). In such cases, bacteria causing the infection can penetrate into the blood stream, start multiplication, travelled to other tissues and organs in the body causing other complications. Blood poisoning can also develop from a simple wound, cut or burn as the body is exposed to the foreign particles (Chang et al .,1856). This can reduce the blood pressure level and ultimately damage important organs like the brain and kidneys. Clinically, this condition is termed as septic shock. It interrupts the normal physiological function of the body by reducing the amount of oxygen and other vital nutrients to the body (Walley et al.,1998) Sepsis is a potentially life-threatening condition caused by the body's immune response to a microbial infection (anehe et al.,2015) immune system normally releases chemicals into the bloodstream to tackle and fight against the pathogens. . In most cases, the most common and prominent causative agent is bacteria. But it may also be fungi, virus or protozoan (Lowy et al.,1998) Common sites for the primary infection are the lungs, brain, urinary tract, skin and abdominal organs (Jonathan et al.,2015). In neonatal sepsis, the bacteriological profile differs significantly between developed and developing countries (Sanghvi et al.,1996) Staphylococcus aureus has evolved as a leading causative agent of sepsis, owing to its propensity to produce deep seated tissue infection and bacteremia . Gram negative isolates like Pseudomonas aeruginosa. Escherichia coli, Serratia spp., Haemophilus influenza and Citrobacter spp., have also been reported in neonatal sepsis. In developing countries, Klebsiella pneumonia is the commonest highly reported bacterial agent causing neonatal sepsis, while group B Streptococcus and Coagulase Negative Staphylococci (CONS) are common in developed countries (Kaistha et al.,2018). However, during the course of time, several changes occurred in the microbial cells making antibiotics ineffective (Lee et al.,2018). The aim of the study to determine prevalence of different pathogenic bacterial species causing human sepsis.

METHODOLOGY

Study design: A cross section study was designed from Centre of Microbiology, Sarhad University and Pathology Department, Leady reading Hospitals (LRH) Peshawar, Khyber Pakhtunkhwa (KP), Pakistan Neonates having septicemia were sampled in LRH Peshawar.

Collection of Samples: The vein puncture site was vigorously cleaned with 70% alcohol and allowed to dry. 8ml of blood was collected and needle was then removed from skin. Adhesive bandage was then applied to the vein puncture site to stop bleeding. The top was removed from blood culture bottles, cleaned with 70% alcohol swab and blood specimens were transferred into blood culture bottles. All the bottles were labeled indicating al the record of patient. The needle and syringe was discarded into sharp container. Finally, the specimens were transferred to Microbiology laboratory for further processing.

Pure culture: For obtaining a pure culture and clear morphology, subculture was performed on Blood Agar MacConkey Agar (MCA) then incubated at 37°C for 24 hours. The same procedure was performed on fresh media for obtaining pure culture.

Morphological and biochemical identification of Bacteria: The isolated bacteria were examined by gram's staining test to differentiate between gram-positive and gram-negative bacteria and their morphology. Further identification of bacteria was made by performing a series of biochemical tests using the taxonomic scheme of Bergey's Manual of Determinative Bacteriology such as Citrate, Oxidase, Catalase and Triple Sugar Iron test were performed.

Disc Diffusion: Technique Disc diffusion method described by Kirby Bauer was used for measuring the in vitro susceptibility pattern. A lawn of pure culture was made on sterile MHA plates and antibiotics discs were placed on the plates alongside with negative control. The plates were kept for 24 hours at 37°C. On the very next day, zones formed due to growth inhibition were calculated in millimeter (mm). Susceptibility (Sensitive, intermediate or resistance) of each drug was measure using the guidelines CLSI.

RESULTS

Collection and screening of Blood samples: In the current study, 121 blood samples were collected from septicemia suspected patients in Lady Reading Hospital, Peshawar. Among 121 blood samples, 43 (35.53%) blood cultures were positive for septicemia. Out of 43 cases of these cultures, 27 (62.79%) showed growth of Gram positive while 16 (37.20%) for Gram negative bacteria. The most common Gram positive isolates were Staphylococcus aureus 19 (70.37%) and Enterococcus spp., 8 (29.62%) (Table 3.1). Among Gram negative isolates were Klebsiella pneumonia 6 (37.5%). Followed by Pseudomonas aeruginosa 4 (25%), Escherichia coli 4 (25%), Serratia 1 (6.25%), and Citrobacter freundii 1 (6.25%) (Table 3.1).

Table 1: Frequency and percentage of isolates from positive cultures

S.No	Bacterial Isolates	Frequency	Percentage
Gram Positive Isolates			
1	Staphylococcus aureus	19	70.37
2	Enterococcus spp	8	29.62
Gram Negative Isolates			
1	Pseudomonas aeruginosa	4	25
2	Klebsiella pneumonia	6	37.5
3	Serratia spp	1	6.25
4	Escherichia coli	4	18.75
5	Citrobacter Freundii	1	6.25

Microscopic Examination (Gram staining): Bacterial isolation on differential and selective media was followed by Gram staining and other biochemical tests for further identification and confirmation. In present study, Gram staining was performed to differentiate between Gram positive and negative isolates. Gram positive species showed purple color while Gram negative showed pink due to changes in peptidoglycan. Based on the results of Gram staining, 27 (62.79%) isolates were identified as Gram positive while 16 (37.20%) as Gram negative, out of the total 43 positive blood samples as mentioned in Table 3.1. The representative picture and pie chart are presented in figure as mentioned in Table 3.1. The representative pictures and pie chart are presented.

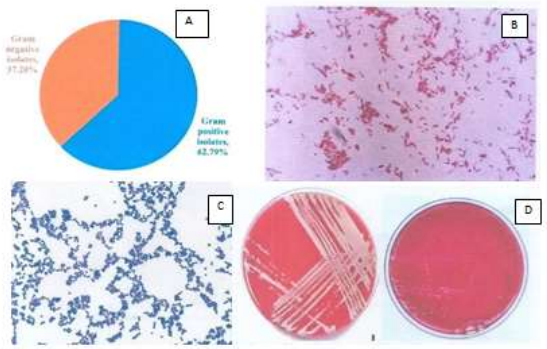

Figure 1 A: Percentage of Gram positive and negative bacterial isolates (B) View of Klebsiella pneumonia under microscope (C) Microscopic view of Staphylococcus aureus (D) Growth of Staphylococcus aureus and Growth of Enterococcus spp.,

Biochemical Tests: Various biochemical tests (catalase, oxidase, citrate and TSI) were performed performed for identification of Gram negative and gram positive and the results are presented in (Table 3.2) and (Table 3.3)

Table 2: Biochemical tests for the identification of gram positive isolates

S.No	Bacteria	Catalase	Citrate	Oxidase	Glucose	Lactose	Sucrose	TSI
1	S.aureus	+	+	-	+	+	+	A/A
2	Enterococcus	-	-	-	+	+	+	A/A

Table 3: Biochemical tests for the identification of Gram negative isolates.

S.No	Bacteria	Cit	Cat	Oxi	Glu	Lac	Sue	H_2S	Gas	TSI
1	E.Coli	-	+	-	+	+	+	-	+	A/A.G
2	P.aeruginosa	+	+	+	-	-	-	-	-	K/K
3	K.pneumonia	+	+	-	+	+	+	-	+	A/A.G
4	Serratia spp	+	+	-	+	+	+	-	-	A/A
5	C. freundi	+	+	-	+	+	+	+	+	A/A, G,H_2S

Abbreviations:Lac=lactose.Glu=glucose, Suc=sucrose, Cit= citrate, Oxi = oxidase, Cat =catalase, TSI = triple sugar iron, A/A, G = acidic/acidic, gas production, k/k = alkaline/alkaline.

Table 4: Percent Sensitivity and resistivity of Gram positive isolates against selected antibiotics.

S.No	Antibiotics	Concentration(ug)	S. aureus		Enterococcus spp.,	
			R	S	R	S
1	Cefoxitin	30	71.73	28.27	-	-
2	Ampicillin	10	90.15	9.85	60.66	39.34
3	Doxycycline	30	70.20	19.80	38.11	61.89
4	Linezolid	30	0	100	0	100
5	Gentamycin	120	56.88	43.12	72.20	27.80
6	Minocycline	30	-	-	14.31	85.79
7	Rifampicin	5	-	-	0	100
8	Teicoplanin	30	-	-	14.98	85.12

Table 5: Percent sensitivity and resistivity of Gram negative isolates.

S.No	Antibiotics	Con	K. Pneumonia		E.coli		P.aeruginosa		Citrobacterspp		Serratiaspp	
1	Ampicillin	10	100	0	100	0	100	0	100	0	100	0
2	Doxycycline	30	84	16	55	45	-	-	0	100	0	100
3	Aztreonam	30	94	6	63	37	56.5	23.5	0	100	-	-
4	Gentamycin	10	84.7	15.3	45	55	34.6	63.4	100	0	0	100
5	Cefepime	30	0	100	100	0	45	55	0	100	0	100
6	Minocycline	30	41	59	54	46	68.5	31.5	0	100	0	100

R= Resistance S= Sensitivy

Antibiotic Resistance: Antibiotic resistance is rising to dangerously high levels throughout the world. Bacteria adopt new resistance mechanisms and become difficult for the professionals to eradicate them. Keeping in view this deadly issue, the antimicrobial sensitivity pattern of different bacterial isolates was studied to find out MDR, XDR and PDR bacterial isolates. The antimicrobial sensitivity pattern of about 43 bacterial isolates was studied in the current research out of which 24 (55.81%) were MDR, 12 (27.90%) were sensitive, 7 (16.27%) were XDR and luckily no PDR isolate was found.

DISCUSSION

In the current study, we determined the bacteriological profile along with their antibiotic sensitivity pattern of 121 clinically suspected cases of Septicemia. Among these samples, 43 were shown as positive culture with blood culture positivity rate of 35.53%. The incidence of Gram positive and negative isolates was 62.79 and 37.20%, respectively. We compared our results with other reports that were representing a high blood culture positivity rate (56%) in septicemia children (Sharma et al.,1987) The frequency of Septicemia in infants differs from area to area. In septicemia, the most common pathogens found in the developing countries vary from those found in developed one. In our research, most common pathogens were Gram negative bacteria followed by Gram K. pneumonia, Serratia and Citrobacter spp while in Gram positive isolates, S. aureus and Enterococcus spp., were found. Gram positive and Gram negative septicemia was encountered in 62.79% and 37.53% of culture positive cases in our study, which is comparable with to a study reported by P jyothi et al, which represents the 41% and 59% for Gram positive and Gram negative isolates, respectively (Jyoth et al.,2003). Antibiotic resistance is currently a global problem for the health professionals. The MDR microbes responsible for neonatal sepsis in developing countries are increasing at an alarming rate. The extensive use of broad-spectrum antibiotics in the world may worsen this situation. Therefore, it is a laborious and hard task to compare the antibiotic resistance among countries because epidemiology of neonatal sepsis is extremely variable (Shatalov et al.,2015). In the current study, I obtained total 43 positive cultures representing 7 common isolates. Among these isolates, 24 (55.81%) were MDR, 12 (27.90%) sensitive,7 (16.27%) were XDR and luckily no PDR isolate was found. All the Gram negative isolates were resistant to ampicillin whereas all the Gram positive isolates were sensitive to linezolid which is comparable to a reported study. Their study reported that out of 1060 isolates, 37.1% were MDR, 13.8% were XDR, and no PDR which is contrary to my results. The predominant Gram negative isolates in my study were K. pneumonia 7 (43.75%) followed by E.coli 4 (25%), P. aeruginosa 4 (25%) Serratia 1 (6.25%) and Citrobacter 1 (6.25%) and while Gram positive isolates were S. aureus 19 (70.37%) and Enterococcus spp., 8 (29.62%). The results of our study are in contrast with the findings of the other researchers, which reported 36.6% cases of E.coli, 29.5% of S. aureus, 22.4% of P. aeruginosa and 7.6% Klebsiella spp., (Al-Otaibi et al.,2006).

CONCLUSION

In the current study a total of 121 samples were collected from suspected septicemic infants and screened for the presence of pathogenic bacteria. Among 121 samples, 43 (35.53%) blood cultures were positive for septicemia. Out of 43 cases, 27 (62.79%) showed growth of Gram positive while 16 (70.37%) and Enterococcus spp., 8 (29.62%). Among Gram negative isolates were K. pneumonia 6 (37.5%) followed by P. aueruginosa 4 (25%), E.coli 4 (25%), Serratia 1 (6.25%) and Citrobacter spp., 1 (6.25%).The S. aureus isolates were resistant to penicillin and cephalosporin family while Enterococcus isolates were resistant to aminoglycosides and penicillin family. The isolates of Enterobacteriaceae were mostly resistant to penicillin and were sensitive to cefepime and gentamycin. In this research, 24 (55.81%) isolates were MDR, 12 (27.90%) were sensitive, 7 (16.27%) were XDR and luckily no PDR isolates were found.

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