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Incidence of Different Bacterial Pathogens Associated with Hospitalized Septic Patients from Bhubaneswar

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The incidence of different bacterial pathogens and their antibiogram profile isolated from septic patients from Bhubaneswar was studied from March 2009 to October 2009. Out of 155 patients 92.26% were culture positive for different bacterial pathogens. Bacteriological analysis of the culture positive cases revealed 27.27% were *Staphylococcus aureus*, *Streptococcus pyogenes*-8.39%, other *Staphylococcus* species-8.39%, *Pneumococcal* species-3.5%, *E.coli*-11.9%, *Klebsiella* species -9.8%, *Pseudomonas* species -4.9%, *Serratia* species-2.8%, *Proteus* species-0.7% and gram positive rods-7.7%. *Staphylococcus, Klebsiella* and *Pseudomonas* were resistant to most of the antibiotics. Bacteriological analysis with septic patients is highly essential for the treatment and management of these patients in this area. This type of study should be continued in other parts of Odisha including more number of patients.

Key words: Sepsis, Bacterial pathogens, Antibiotic sensitivity.

Sepsis is an increasingly common cause of morbidity and mortality, particularly in elderly, immune-compromised and critically ill patients. The term Sepsis popularly implies a clinical response arising from infection[1]. Sepsis is defined as an infection that triggers a particular Systemic Inflammatory Response Syndrome (SIRS)[2]. Invasion of the body by disease causing organism that become established, multiply and produce symptoms. Bacteria and viruses cause most diseases, but diseases are also caused by other organisms, like protozoa and other parasites[3]. A less common route of entry is through the skin, either by contamination of an open wound or by penetration of the intact skin surface[4]. Symptoms of sepsis are usually nonspecific and include fever, chills, and constitutional symptoms of fatigue, malaise, anxiety, or confusion. These symptoms are not limited to infection and may be seen in a variety of non-infectious inflammatory conditions. According to the guidelines established by American College of Chest Physicians: Society of Critical Care Medicine Consensus Conference, a patient is diagnosed with SIRS when he or she presents with two or more of the following criteria: (1) temperature greater than 38°C or less than 36°C;(2) an elevated heart rate greater than 90 beats per minute; (3) respiration rate greater than 20 breaths per minute, as indicated by a $PaCO_2$ of less than 32mm Hg; and (4) an alternation in the white blood cell count greater than 12,000/cu mm, a count less than 4,000/cu mm; or the presence of more than 10 percent immature neutrophils[1]. Severe sepsis is defined as sepsis associated with organ dysfunction, hypo perfusion abnormality or sepsis induced hypotension[1]. Barely more than 50% of the patients with severe sepsis admitted to the hospitals survive. This unacceptable high mortality can only be reduced if there is greater awareness and understanding of the condition[2].

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Sepsis causes millions of deaths globally each year[5]. The overall incidence of wound sepsis in India is from 10-33%. Western studies indicate the range to be between 3-10% with an average of 5%. Urinary tract infection is reported to be highest (42%), followed by wound infection (23.8%) and respiratory tract infection (10.5%)[6]. Septic shock develops in about 40% of sepsis patients. Death due to septic shock increased to 82.6% from 1979 to 1997 in US, with approximately 4.2 deaths per 100,000 populations[7]. Around 750,000 cases of sepsis are diagnosed per year and between 28 to 50 percent of those diagnosed die[8-9]. The total hospital cost associated with the care of patients which included severe sepsis cases was $16.7 billion[8]. This is due to relative resistance of antibiotics which is difficult to treat. As, more virulent strains with capacity to adapt quickly to changing environment make the pathogens acquired in the hospital a matter of concern[6]. Various studies across the globe have been consistent enough to show a predictable bacterial profile in the wound infections; which makes an important observation for a clinician who intends to start empirical treatment to his patients, while laboratory culture reports are awaited[6]. Selection of appropriate antibiotics that can cover both gram positive and gram negative micro-organisms at this phase is usually empiric. This selection depends on factors like epidemiological evidences, antibiotic resistance patterns and probable infective cases[10]. A few studies have been reported from Odisha on sepsis patients. Therefore, the present study has been envisaged to document the spectrum of bacterial pathogens isolated from wounds, abscess, burns, ulcers, gangrene etc of sepsis patients along with its antibiogram profile.

MATERIALS AND METHODS

Bacteriological analysis

During study period (March, 2009 to October, 2009) tissue fluid (fresh pus) from different age group of patients were collected by sterile swabs in sterile vials bearing the patients name, age and were transported to microbiology division of Regional Medical Research Centre (RMRC), Bhubaneswar. The samples were immediately inoculated on macconkey, blood Agar and nutrient Agar. The plates were inoculated at 37°C for 24hrs. Blood Agar plates were incubated in aerobic and anaerobic conditions. Significant colonies were picked up for gram's staining and finally biochemical tests were done for confirmation of bacterial species. Identification of isolates were done based on colony morphology, motility test, catalase, coagulase and biochemical test like urease test, triple sugar iron agar (TSI), mannitol motility test, iodole test, citrate utilization test, oxidase test etc.

Antimicrobial susceptibility

Antibiotic susceptibility analysis was performed by modified Kirby Bower Disk diffusion technique (1966) with commercially available antibiotic disc. Characterization of strains as susceptible, intermediately resistant or resistant was done as based on the size of the inhibition zone according to the manufacturer's instruction which matched the interpretive criteria recommended by WHO[11]. Antibiotics used in this study were kanamycin (K, 10µg), bacitracin (B, 10µg), penicillin (P, 10µg), cefuroxime (Cu, 30µg), trimethoprim (Tr, 30µg), piperacillin (Pc, 100µg), rifampicin (R, 30µg), fusidic acid (Fc, 10µg), co-trimoxazole (Co, 25µg), streptomycin (S, 10µg), ciprofloxacin (cf, 5µg), tetracycline (T, 30µg), gentamicin (G, 10µg), norfloxacin (Nx, 10µg), erythromycin (E, 15µg), neomycin (N, 30µg), ampicillin (A, 10µg), nalidixic acid (Na, 30µg), chloramphenicol (C, 30µg) and furazolidone (Fr, 50µg).

RESULTS

Out of total 155 cases 143 (92.26%) were culture positive 12(8.4%) from respiratory system infection, burn wound- 12(8.4%), accidental infection-39(27.3%), abscess- 22(15.4%), ulcer-27(18.9 %), soft tissue infection- 30(20.97%), gangrene-5(3.5%), peritonitis- 1(0.7%) and pyelonephritis-2(1.4%). From 143 culture positive samples; *Staphylococcus aureus* were isolated 39 (27.3%), coagulase negative *Staphylococcus spp*. 29 (19.58%), *Streptococcus pyogenes* 17 (8.39%), other *Streptococcus spp*. 12 (8.39%), *Pneumococcal spp*. 5 (3.5%), *E.coli* 17 (11.9%), *Klebsiella spp*. 14(9.8%), *Pseudomonas spp*. 7 (4.9%), *Serratia spp*. 4 (2.8%), *Proteus spp*. 1 (0.7%) and gram positive rods 11 (7.7%) respectively (Table 1).

Table 1. Spectrum of different Bacterial pathogens isolated from Septic patients

S. No	Clinical cases	Total cases	Culture positive for bacteria	A	B	C	D	E	F	G	H	I	J	K
1.	Accidental infections	42	39(27.3)	20.5	25.7	15.4	5.1	2.6	7.7	0	2.6	7.7	5.1	7.7
2.	Burn infection	12	12(8.4)	25	8.3	8.3	8.3	0	0	0	8.3	0	41.7	0
3.	Soft tissue infection	33	30 (20.9)	16.7	20	0	13.3	3.3	13.3	3.3	3.3	6.67	0	20
4.	Abscess	22	22 (15.4)	27.3	22.7	0	4.5	0	22.7	0	0	13.6	0	9.1
5.	Ulcer	24	27 (18.9)	29.6	18.5	11.1	7.4	3.7	14.8	0	0	14.8	0	0
6.	Respiratory system infection	14	12 (8.4)	25	16.7	8.3	0	16.7	8.3	0	8.3	16.7	0	0
8.	Peritonitis	1	1(0.7)	100	0	0	0	0	0	0	0	0	0	0
9.	Pyelonephritis	2	2 (1.4)	100	0	0	0	0	0	0	0	0	0	0
10.	Gangrene	5	5(3.5)	60	0	0	40	0	0	0	0	0	0	0
	Total	155	143(92.3)	27.3	20.3	7.7	8.4	3.5	11.9	0.7	2.8	9.8	4.9	7.7

A) *S. aureus* B) Other *Staphylococcus* spp. C) *S. pyogenes* D) Other *Streptococcus* spp. E) *Pneumococcal* spp.
F) *E. coli* G) *Proteus* spp. H) *Serratia* spp. I) *Klebsiela* spp. J) *Pseudomonas* spp.
K) Gram +ve rods

Table 2. Biochemical characteristics of different Bacterial pathogens isolated from Septic patients

	Urease	Glucose	Lactose	H_2S	Gas	Citrate	Manitol	Motility	Indole	Catalase	Oxidase
Staphylococcus aureus (coagulase positive)	+	+	+	-	-	-	+	-	+	+	-
Staphylococcus spp. (coagulase negative)	+	+	+	-	-	-	+	-	-	+	-
Streptococcus pyogenes	+	+	+	-	-	-	+	-	+	-	-
Other Streptococcus spp.	+	+	+	-	-	-	+	-	+	-	-
Pneumococcal spp.	+	-	+	-	-	+	+	-	+	+	+
E. coli	-	+	+	-	+	-	+	+	+	+	-
Proteus spp.	+	+	-	+	v	v	-	+	v	+	-
Serratia spp.	-	+	-	-	v	+	+	+	-	+	-
Klebsiella spp.	+	+	+	-	+	+	+	-	v	+	-
Pseudomonas spp.	+	-	-	-	-	-	+	V	-	+	+
Gram positive Rods	-	v	+	-	-	v	+	v	+	+	-

Table 3. Resistance Profile (%) of different Bacterial pathogens isolated to different antibiotics

Antibiotics*Bacteria	K	B	P	Cu	Tr	Pc	R	Fc	Co	S	Cf	T	G	Nx	E	N	A	Na	C	Fr
Staphylococcus aureus *(coagulase positive)*	92.3	87.2	89.7	89.7	74.4	87.2	74.4	41.1	51.3	58.9	97.4	48.7	38.5	97.4	71.8	82.1	94.8	100	28.2	97.4
Staphylococcus *(coagulase negative)*	100	96.6	86.2	100	75.9	86.2	86.2	79.3	58.6	41.4	82.8	68.9	72.4	89.7	89.7	82.8	75.9	93.1	68.9	96.6
Streptococcus pyogenes	63.6	72.7	81.8	90.9	72.7	81.8	54.6	90.9	18.2	36.5	100	36.5	36.5	100	81.8	63.6	81.8	100	72.7	100
Other *Streptococcus Spp.*	83.3	91.7	100	75	91.7	91.7	83.3	100	91.7	41.7	66.7	66.7	66.7	91.7	100	58.3	58.3	100	100	91.7
Pneumococcus	100	100	100	100	60	80	100	100	40	40	80	80	40	100	100	20	80	100	60	100
Gram positive Rods	90.9	100	90.9	81.8	72.7	100	36.5	36.5	72.7	63.6	90.9	27.3	72.7	90.9	90.9	90.9	90.9	90.9	100	100
Proteus	100	100	100	100	0	100	100	100	100	0	100	100	100	100	100	100	100	100	100	100
E. coli	94.1	100	100	100	88.2	94.1	58.8	100	29.4	52.9	100	88.2	64.7	82.4	100	41.2	100	100	64.7	76.5
Klebsiella	100	100	100	100	92.9	100	78.6	100	28.6	92.9	100	85.7	92.9	78.6	100	85.7	100	100	42.9	50
Pseudomonas	100	100	100	100	100	100	42.9	100	100	100	57.2	100	85.7	71.4	100	100	100	100	100	100
Serratia	50	100	100	50	75	100	75	100	25	25	25	25	25	50	100	25	100	100	50	75

*Kanamycin-K, Bacitracin-B, Penicillin-P, Cefuroxin-Cu, Trimethoprim-Tr, Piperacillin-Pc, Rifampin-R, Fusidic acid-Fc, Co-trimoxazole-Co, Streptomycin-S, Ciprofloxacin-Cf, Tetracycline-T, Gentamycin-G, Norfloxacin-Nx, Erythromycin-E, Neomycin-N, Ampicillin-A, Nalidixic acid-Na, Chloramphenicol-C, Furazolidone-Fr.

The incidence of positive cases was found more among male (70.9%) than the female (29.03%). Most of the infections were associated with single pathogens. In accidental infections, ulcer and respiratory system infection multiple bacterial pathogens were isolated from few patients. Important etiological agent found in most infections was *Staphylococcus spp*. especially *Staphylococcus aureus* isolated from accidental infections were primarily *Staphylococcus aureus* and b- hemolytic *Streptococcus pyogens*. Among the 39 *Staphylococcus aureus* only 20 strains showed b- hemolysis which seems to be virulent. The biochemical characteristics of different bacterial pathogens isolated from septic patients were described in Table 2.

Antimicrobial resistance status of microorganisms isolated is shown in Table 3. These microorganisms were exposed to antibiotics in order to detect the resistance limit of them to common antibiotics. As shown, *Staphylococcus aureus* obtained were resistant to kanamycin, bacitracin, penicillin, cefuroxime, trimethoprim, piperacillin, rifampicin, ciprofloxacin, norfloxacin, erythromycin, neomycin, ampicillin, nalidixic acid and furazolidone. Similar resistance patterns were obtained for other coagulase negative *Staphylococcus spp. Streptococcus pyogens* were resistant to penicillin, cefuroxime, piperacillin, fusidic acid, ciprofloxacin, norfloxacin, erythromycin, ampicillin, nalidixic acid and furazolidone. However other *Streptococcus spp.* were resistant to kanamycin, bacitracin, penicillin, trimethoprim, piperacillin, rifampicin, fusidic acid, co-trimoxazole, norfloxacin, erythromycin, nalidixic acid, chloramphenicol and furazolidone. *Pneumococcus spp.* were resistant to kanamycin, bacitracin, penicillin, cefuroxime, piperacillin, rifampicin, fusidic acid, ciprofloxacin, tetracycline, norfloxacin, erythromycin, ampicillin, nalidixic acid and furazolidone. *E.coli* were resistant to kanamycin, bacitracin, penicillin, cefuroxime, trimethoprim, piperacillin, fusidic acid, ciprofloxacin, tetracycline, norfloxacin, erythromycin, ampicillin and nalidixic acid. *Klebsiella spp*. were resistant to kanamycin, bacitracin, penicillin, cefuroxime, trimethoprim, piperacillin, rifampicin, fusidic acid, streptomycin, ciprofloxacin, gentamicin, erythromycin, neomycin, ampicillin and nalidixic acid. *Pseudomonas spp.* were resistant to kanamycin, bacitracin, penicillin, cefuroxime, trimethoprim, piperacillin, fusidic acid, co-trimoxazole, streptomycin, tetracycline, gentamicin, erythromycin, neomycin, ampicillin, nalidixic acid, chloramphenicol and furazolidone. *Serratia spp.* were resistant to bacitracin, penicillin, trimethoprim, piperacillin, rifampin, fusidic acid, erythromycin, ampicillin, nalidixic acid and furazolidone. *Proteus spp*. were resistant to trimethoprim and streptomycin. Gram positive rods were resistant to kanamycin, bacitracin, penicillin, cefuroxime, piperacillin, ciprofloxacin, norfloxacin, erythromycin, neomycin, ampicillin, nalidixic acid, chloramphenicol and furazolidone. Therefore, it was observed that relatively all the pathogens isolated were resistance to most of the common antibiotics used.

DISCUSSION

Sepsis is a major health problem in many countries. The present study has been envisaged that the common bacterial infections were mainly due to *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas*, etc. Similar prevalence of pathogens has been isolated from infected patients admitted to Dhiraj general hospital, Baroda during 2013[6]. Verma (2012) has reported that *Staphylococcus aureus* was predominant followed by *Klebsiella, Pseudomonas, E.coli and Proteus* was isolated from outpatients at Pt. Jawaharlal Nehru memorial medical college and Dr. B.R.A.M. Hospital, Raipur[13]. Panahi et.al., (2008) has reported that Staphylococcus *aureus*, *Klebsiella*, *Pseudomonas*, etc were isolated from Septicemia patients admitted to ICU in Tehran, Iran[14]. Septic cases varied with different sex as it was found relatively more among male population than the female. Similar conclusions were drawn in the research done in United States[7].The predominance of mono-microbial infections observed in this study has been substantiated by a prospective study done in Banaras Hindu University, Varanasi stating that chronic wounds tend to show mono-microbial infections[12]. Despite years of research, scientists have not yet succeeded in developing a medicine that specifically targets the aggressive immune response that characterizes sepsis. Thus the control of the pathogens heavily relies on intensive use of various antibiotics. However, as a result of

increasing use of antibiotics, the pathogenic bacteria become more easily resistant to a wide range of these drugs[15]. For example, *Staphylococcus*, *Klebsiella* and *Pseudomonas* obtained in this study were resistant to most antibiotics which correlates with the findings of Basu *et al*.,[12]; Agnihotri *et al*.,[16]; and Karia *et al.*,[6].

This type of study should be continued for longer period in different parts of Odisha including more number of patients to look for the spectrum of bacterial infections associated with septic patients. High clinical suspicion and appropriate microbiological tests is also essential for early diagnosis and proper administration of antibiotics to reduce motility rate.

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