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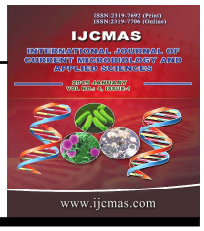

Original Research Article

Incidence and antimicrobial resistance among potential nosocomial bacteria isolated from indoor environment of hospital

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Keywords

Nosocomial infections, antimicrobial resistance, indoor environment of hospital

A B S T R A C T

Nosocomial infection is major cause of significant morbidity and mortality worldwide. The patients those are in intensive care, at higher risk of developing nosocomial infections with resistant strains. This study was planned to isolation and characterization of drug resistance among bacteria causing infection in hospitalized patients. One hundred and sixty five patients admitted in various wards were included in this study. A total of 165 clinical samples were collected for these patients. The samples comprised of blood, urine, wound swab, pus sputum and various aspirated body fluids. The percentage of ICUs samples is more than other parts of the hospital. ICUs sample percentage was 86.84% and percentage from other parts of the hospital was 13.16%. A total of eighty one bacterial isolates were obtained from 165 patients those admitted in hospital. The most common organism came to be *E. coli.* The antimicrobial sensitivity was done by Kirby and Bauermethod in all isolates. The sensitivity pattern of *Klebsiella pneumoniae* showed that 87.5% of the isolates were sensitive to polymyxin B, followed by 75% to amikacin, 62.5 % sensitive to both netimycin and Ciprofloxin. As much as 43.7% of Staphylococcus were resistant to amikacin and cefaperazone plus sulbactum while 50% percent of them were sensitive to netimycin and polymyxinB. However, 56.3% were sensitive to ceftazidime. Hospital authority should follow the guidelines to control of nosocomial infection moreover monitoring and trends of pathogen play important role in to reducing emerging antimicrobial resistant in pathogens.

Introduction

Nosocomial infections are continued to be an important cause of morbidity, mortality, prolonged hospital stay and extra financial burden to patients (Roka *et al.,* 2006). The type of infection vary, the most common infection being urinary tract infection and pneumonia accounted for 60% of total hospital infections. Different bacterial species have been isolated from various hospitals across the world. Some of them are resistant to number of antimicrobial agents, they are opportunistic pathogens and hence pose a challenge to patients especially those who are immunocopromised (Markovic, 2009). Nosocomial infection is a crucial problem to patients especially in high department such as intensive care unit (ICU). The patients those admitted in ICU at high risk as result mechanical ventilation, prolonged catheterization and improper antimicrobial treatments.

Materials and Methods

The study was conducted in the hospital in the department of Microbiology in Dayanand Hospital, Ludhiana from June 2011 to May 2012. Official request were made to and approval taken from administrative head of the hospital. There are separate wards of all medical and surgical specialties for in patients and has a good number of consultants in various field of medicine. A total of 07 wards were selected for evaluation of incidence of infection. These were as Stroke ICU, pediatric ICU, Medical I C U, Surgery ICU, Neurology Ward, Medical Ward and Neurosurgery ward. A total of 165 patients were selected for this study. The patients which were newly admitted to hospital were considered for study. They were monitored from the day of admission to time of discharge.

Sample collection from patients

The site of infection was classified according to type of specimen as being sent to microbiology laboratory for investigation. When a sample was taken from wound and laboratory culture result was positive, site considered surgical site infection, whereas sample involved was urine with significant bacterial level the infection acquired is considered as urinary tract infection. While a sample cultured from sputum and blood produced positive colonies reflected respiratory and bloodstream infection respectively.

Blood culture

Five ml blood was withdrawn from vein of the patients when they develop fever without initiation of antibiotics treatment. Sterility of the skin was ensured by application of methylated sprit. The collected blood was directly incubated in blood culture bottles containing 50 ml of glucose broth and incubated at 37°C for one week. The bottles were inspected for turbidity every two days of interval. If bottle showed turbidity, the sub cultured was made from them on blood agar as well as on MacConkey agar plate. No growth after one week determined as negative culture.

Identification of organism

All clinical samples were collected from patients during their stay in hospital and grown on MacConkey agar, chocolate agar, and blood agar at 37°C and further studied on the basis of colony morphology and gram staining. Physical characterization such as size, shape, surface of colony, edges, color. Opacity and hemolytic properties was studied. A representative colony from pure culture as subjected to various biochemical tests which performed according to procedure describe by Collee *et al.* (1996).

Antibiotic Sensitivity Test

Disc diffusion method was used for evaluating the susceptibility of isolate to various antimicrobial agents. Antibiotics with commercial prepared were tested on all isolates on Muller-Hinton agar plate. The results were interpreted as per guidelines of CLSI (2009).

Result and Discussion

One hundred and sixty five patients admitted in various wards were included in this study. Among these 121 were male and 44 female. Statistical analysis showed that male and female patients were suffered with illness and admitted for treatment (Table 1). The age group and underlying illness was also ruled out. The distribution of admission in hospital and age group was not equally

distributed. It was observed that age was directly related to the admission. Older the age, higher was the proportion of patients admitted to the hospital. The comparison between samples collected from different ICUs and other parts of the hospital.

ICUs sample percentage was 86.84% and percentage from other parts of the hospital was 13.16%. A total of 165 clinical samples were collected for these patients. The samples comprised of blood, urine, wound swab, pus sputum and various aspirated body fluids. It was well justified in the given graph that two sources such as blood and urine culture had higher percentage of specimen which were 32.73% and 23.64% respectively. Besides that, Cather tip c/s, CSF, Sputum and Wound swab had same percentage of specimen which was 4.85%. Sources like as ET Sec and Tracheal secretion had percentage of specimen were 8.48% and 6.67%. Pleural Fluid and Pus had 4.24% and 3.64% of specimen respectively. The source like as ascitic fluid had least number of percentage of specimen which was only 1.21%.

Identification of pathogenic bacteria

A total of eighty one bacterial isolates were obtained from 165 patients those admitted in hospital. These were grown on different media and gram's stain was performed to distinguish gram positive and negative bacteria. Out of 165 samples, 81 (49.09%) grew organisms. The highest incidence of organism growth was found to be *E. coli* (23/81; 28.4%), followed by *Staphylococcus* (16/81; 9.75%) and *Acinetobacter* (12/81; 14.81%). There were 54 samples of blood. In 14 (25.93%) of these organisms was seen grown. The most common organism came to be *E. coli* and *Staphylococcus* (each 5/14; 35.71%). This was followed by *Acinetobacter* (2/14; 14.29%). The second most common specimen was urine which was 39 in number. Of 39, organism grew in 23 (58.97%) samples. The most common organism was *E. coli* (9/23; 39.13%) followed by *Candida albinos* (7/23; 30.43%). Out of a total of 165 specimens, organisms were developed in 81 (49.09%) samples. The most common organism was *E. coli* (23/81; 28.40%), followed by *Staphylococcus* (16/81; 19.75%) and *Acinetobacter* (12/81; 14.81%). There is the brief elaborated data of 8 organisms in all different type of sources of sample.

It was well justified that 23 pathogenic bacteria *E. coli* was isolated from the sample which was in higher number. After this 16 *staphylococcus* bacteria isolated from the sample. 12 number of *Acinetobacter* bacteria were isolated from all the samples. Apart from this, number of pathogenic bacteria's *Klebsiella* and *Candida Alb* was 8 in the equal numbers. Besides that 7 *Pseudomonas*, 5 *Enterococcus* and 2 *Asper Flavus* bacteria's were isolated from the all samples.

Overall antimicrobial resistance

There were total 81 organisms in the sample of the study. The highest proportion is 70.37 percent of these were sensitive to polymyxin B, followed by 58.02 percent to piperacillin plus tazobactum, 55.56 percent to cefaperazoneplus sulbactum and 54.32 percent to netimycin. Sensitivity pattern showed that 48.15 percent of the isolates were sensitive to amikacin, followed by 45.68 percent to gentamycin and 40.74 percent of the isolates were sensitive to Ciprofloxin. There were only 22.22 percent of the isolates which were sensitive to cefotaxime while 27.16 percent of the isolates were sensitive to ceftazidime. The antimicrobial resistance pattern in all species which was described in two ways one is

sensitive and another one is resistant (Table 1). It was well elaborated with number isolates in different antimicrobial agents. Sensitivity and resistant of gentamycin was 38 and 35 respectively. As like as in Netimycin Sensitivity and resistant was 43 and 30 respectively. Apart from this in other antimicrobial agents such as ceftazidime, cefotaxime, Ciprofloxin had less sensitivity than resistance which was 20 and 45, 18 and 49, 32 and 40 respectively. Antimicrobial agents like as imipenem and Amikacin had high sensitivity and had less resistance which was 49 and 30, 40 and 38 respectively. Another antimicrobial agent for instance, Cefaperazone + Sulbactum, Piperacillin + Tazobactum, shown higher sensitivity and less resistance which had number of isolates 45 and 35, 49 and 30 respectively. In the whole graph Polymyxin B antimicrobial agent had higher sensitivity than another antimicrobial agent which was 60 and had lesser resistance which was 20 numbers of isolates.

In the present study, about 24 % of patients who developed nosocomial infections were admitted in the hospital outside prior to admission to hospital. Incidence of infection was directly related to the hospital stay. The most common risk factor of nosocomial infection was prolonged mechanical ventilation, which was found in 31.52 percent of the cases, followed by renal failure (14.55%), Hemodialysis (9.09%) and Aspiration (7.27%). Coma and TB were seen only in 1.82 and 1.21 percent of patient. Out of 165 patients, who had nosocomial infections, 41.21 percent got improved and shifted to respective wards. As much as 39.39 percent left the hospital with LAMA while only 19.39 percent of them expired. In the present study, male patients suffered with illness and admitted for treatment to the significantly higher extent (73.33%) compared to the females (26.67%). Age was directly related to the admissions in hospital for treatment.

Out of total samples of 165, the highest proportion was of blood (32.73%), followed by urine (23.64%) and ET sec (8.48%). The lowest proportion was of Ascitic fluid (1.21%), followed by Pus (3.64%). A classical study done at department of microbiology Indra Gandhi Medical college and research institute Pondicherry, India the author showed in their result that urinary tract infection (UTI) can be caused by gram negative bacteria such as *E COLI, Klebsiella species*, *Enterobacter species* and gram positive bacteria like enterococcus species and *staphylococcus saprophticus E. coli* is most common agents in urinary tract infection (Niranjan *et al.,* 2014). In the present study 81/165 (49.09%) organisms were found. The most common organism was *E. coli* (23/81; 28.40%), followed by *Staphylococcus* (16/81; 19.75%) and *Acinetobacter* (12/81; 14.81%). The present study finds that of 39 urine specimens, organism grew in 23 (58.97 %) samples.

The organism found in urine specimen is 23 out of total 81 organisms i.e. 28.40 percent. Walter *et al.* (1998) finds nosocomial urinary tract infection has been responsible for 35-40 percent of all hospitalized infections. Walter ES concluded this epidemic nosocomial bacteriuria due to use of inadequate disinfected instruments, unsterile solution used for multiple patients, contaminated disinfectants in catheter insertion trays. Stamm *et al.* (1991) reflected that epidemic of nosocomial urinary tract infection transmitted through hospital staff from one catheterized patient to another. Hooton *et al.* (1981) found that females are prone to nosocomial urinary tract infection with comparison of males by approximately two fold in each decade of life.

The major organism found in urine specimen was *E. coli* i.e. 9/23 (39.13%). The second major organism in urine specimen was *Candida albicans* (7/23; 30.43%). Beyene and Tsegaye (2011) reported that the most common pathogen isolated was *E. coli* (33.3%), *Klebsiella* (19%) and *Staphylococcus saprophticus* (14.3%). Chanshckhar *et al.* (2006) showed that urinary tract infection was mainly *E. coli* (59.4%), *Klebsiella* (8.1%). Ouno *et al.* (2013) found that among urinary tract infections the predominant bacteria was *E. coli* accounted for 47.5%, followed by Coagulase negative *Staphylococcus* (22.5%) and *Klebsiella* (10%).

In the present study there were 54 samples of blood. In 14 (25.93%) of these organisms was seen grown. The most common organism came to be *E. coli* and *Staphylococcus* (each 5/14; 35.71%), followed by *Acinetobacter* (2/14; 14.29%). Pittel (1994) recorded that bloodstream infection is important cause of morbidity and mortality in hospitalized patients. Mermel *et al.* (2011) noted that the insertion bacteria in blood stream due to rise of external devices such as central venous catheter which is widely used in health care sector. The central veins catheter used for administration of intravenous fluid, medication blood as well as providing hemodialysis access and hemodynamic monitoring. These devices can be left in place for several days. Blood stream infection can occur when microorganism are colonized on external surface of the device and entered in blood stream causing infection.

Pittel and Wenzel (1994) noted that in appropriate empirical antimicrobial therapy is responsible of death in patients having blood stream infection. Barca *et al.* (1999) studied nosocomial bacteremia in patients having defective immune system. Result showed that 77% cases have bacteremia. Gram positive bacteria were frequently isolated and accounted for 53% episodes of bacteremia while gram negative bacteria responsible for 34% and 48%. A similar study conducted by Wisplinghoff *et al.* (1978) on hospital acquired bacteremia on 2340 patients. The result showed that high mortality due to Coagulase negative *Staphylococci* (33.4%) and other gram positive organism encountered were Methicillin sensitive and resistant *Staphylococci* aureus 22.8% and 17.7% respectively.

Bergjan *et al.* (2001) studied bloodstream infection in patients who undergone bone marrow transplants. A total of 138 patients had single organism, 39 patients had 2 pathogens in their blood and 12 patients had bacteremia due to 3 or more organism in blood samples. Result showed that 250 isolates were identified, out of which 155 (62%) were gram positive bacteria and 95 (38%) were gram negative. Among gram negative *E. coli*, *Pseudomonas* and *Klebsiella* spp. Were frequently isolated while in gram positive bacteria *Streptococcus* (49%) was predominant followed by Coagulase negative *Staphylococcus* (42.5%), *Enterococcus* (7%) and Bacillus and *Corynebacterium* species accounted for 1.5%.

Central venous catheter related bloodstream infection was studied by Velasco *et al.* (2000) the study showed that 56.9% of bloodstream infection due to central venous catheter. The predominant organisms in this study were Coagulase negative *Staphylococcus* (19.5%) followed by *Staphylococcus aureus*, *Pseudomonas aeruginosa* (12%) and *Staphylococcus pyogenes* (7.4%). In the present study, Out of 8 wound swab specimen, 6 (75%) seen growth of organism. *Acinetobacter* and *staphylococcus* were the most common

organisms (each 25%), followed by *E. coli* and *Klebsiella* (each 12.50%). The similar study was conducted for 1 year from January 2008 to December 2008 in all 4 surgical unit of Liaquat University hospital Hyderabad which caters to patients from low socioeconomic status pus culture and sensitivity report ware collected prospectively from hospitalized patients who developed post operated wound infection during the study period 112 pus culture and sensitivity reports were analyzed E. coli 68 (60.7%) is most common organism Ali Syed Abad *et al.* (2009).

Sensitivity Pattern

The present study found that as high as 22/23 (95.65%) of the *E. coli* isolates were sensitive to polymyxin B, 91.30 percent of isolates were sensitive to imipenem. Only 8.70 percent of isolates were sensitive to cefotaxime and the sensitivity was seen in 13.04 per cent of the isolates to ceftazidime. *Klebsiella pneumoniae* showed that 87.50 percent of the isolates were sensitive to polymyxin B, followed by 75.00 percent sensitive to Amikacin, 62.50 percent sensitive to both netimycin and Ciprofloxin and 50.00 percent were sensitive to imipenem and Cefaperazone plus sulbactum. There were 37.50 percent of the isolates which were sensitive to ceftazidime and piperacillin plus tazobactum, while only 25.00 of them were sensitive to cefotaxime.

In the present study, the sensitivity pattern of *Pseudomonas aeruginosa* showed that 85.71 percent of the isolates were sensitive to polymyxin B and Netimycin, followed by 57.14 percent of isolated sensitive to Gentamycin and Cefaperazone plus sulbactum while 42.86 percent of isolates were sensitive to ceftazidime, amikacin, Ciprofloxin and piperacillin plus tazobactum. Only 28.57 percent of the isolates were sensitive to both imipenem and cefotaxime.

Joanne and Ronald *et al.* (1998) found that only 4% of gram positive bacteria were sensitive to piperacillin and tobramycin while 44% of gram negative bacteria showed resistance against above antimicrobial agents. Sixty two percent of Coagulase negative *Staphylococcus* was susceptible to Methicillin and all were resistant to Vancomycin. Antimicrobial resistance pattern showed that 50% *E. coli* and *Klebsiella* were susceptible to piperacillin but Pseudomonas showed resistance to majority of antimicrobial agents including imipenem. Beyene G and Tsegaye *et al.* (2011) found that both gram negative bacteria showed 100% resistant to ampicillin and amoxicillin. Resistance against ceftriaxone, gentamycin and chloramphenicol also recorded in rest of pathogens. Keen *et al.* (2010) found that *Pseudomonas* species were resistant to antimicrobials like amikacin (85.18%), gentamicin (89.22%), ciprofloxacin (78.81%), carbenicillin (88.26%), tobramycin (87.52%) and ceftazidime (79.09%). And *S. aureus* were highly resistant to amoxicillin (69.04%), erythromycin (75.27%), and Netilmicin (77.75%); and 24% of isolated *Staphylococcus aureus* were methicillin resistant.

It may be concluded from the study that incidence of infection is directly related to the hospital stay. The most common risk factor of Nosocomial infection is prolonged mechanical ventilation. Proper infection control masseurs can reduce hospital infection and prolonged survival of hospitalized patients. Hospital authority should follow the guidelines to control of nosocomial infection moreover monitoring and trends of pathogen play important role in to reducing emerging antimicrobial resistant in pathogens.

Table.1 Sensitivity pattern of antibiotics in all species

antibiotic	No.	Sensitive		Resistant		Intermediate	
	(out of 165)	No.	%age	No.	%age	No.	%age
Gentamycin	81	37	45.68	36	44.44	8	9.88
Netimycin	81	44	54.32	31	38.27	6	7.41
Ceftazidime	81	22	27.16	45	55.56	14	17.28
Imipenem	81	47	58.02	31	38.27	3	3.70
Cefotaxime	81	18	22.22	48	59.26	15	18.52
Amikacin	81	39	48.15	37	45.68	5	6.17
Ciprofloxin	81	33	40.74	41	50.62	7	8.64
Cefaperazone+Sulbactum	81	45	55.56	33	40.74	3	3.70
Polymyxin B	81	57	70.37	21	25.93	3	3.70
Piperacillin+Tazobactum	81	47	58.02	29	35.80	5	6.17
Total	810	389	48.02	352	43.46	69	8.52

Figure.1 Percentage of samples collected from all sources in hospital

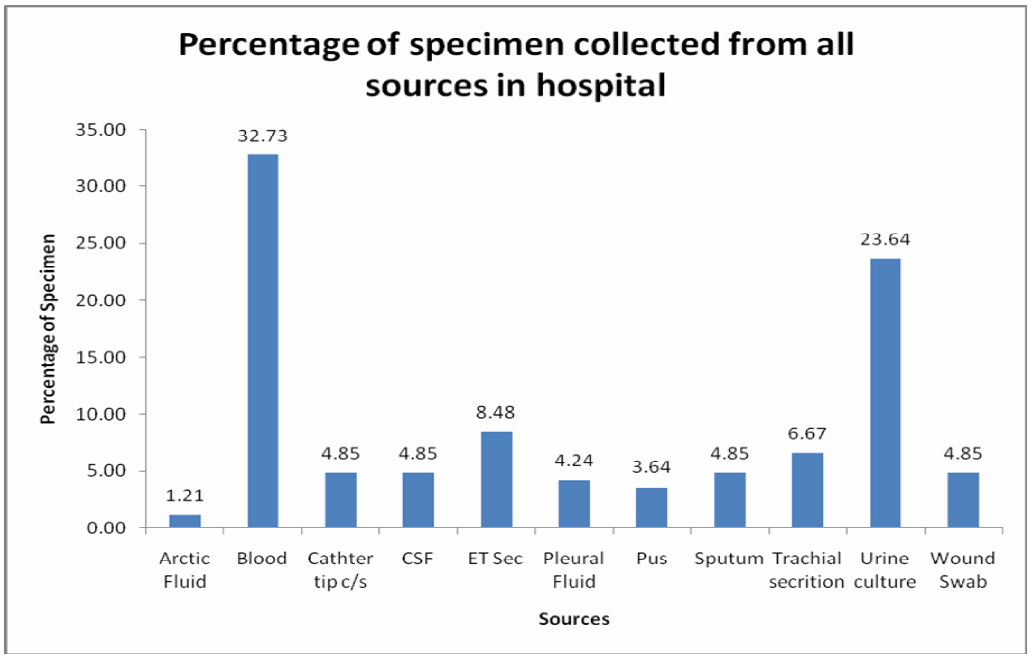
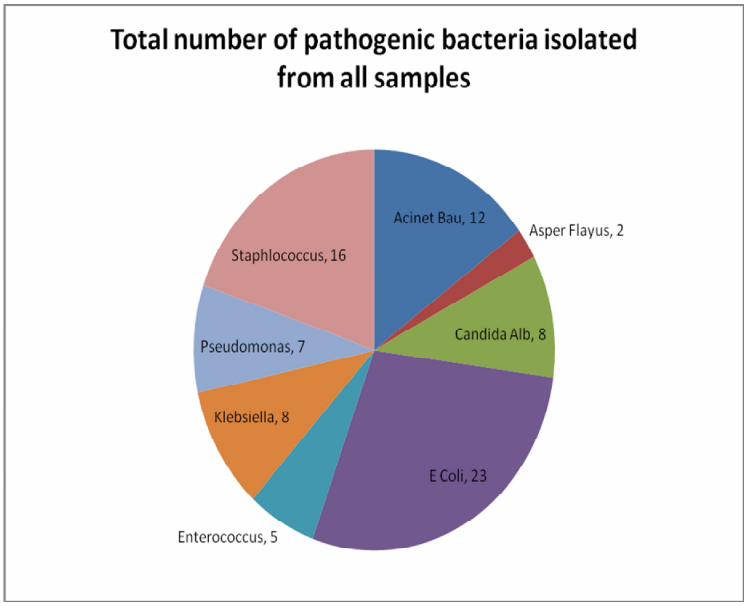


Figure.2 Frequency of pathogenic bacteria isolated from all clinical samples



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