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INCIDENCE OF NOSOCOMIAL INFECTIONS IN HOSPITALIZED PATIENTS

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Abstract:
The term hospital acquired infection or nosocomial infection are applied to infections developing in hospitalized patients. NI are inherent and inseparable dangers of modern therapy, especially for the critically ill patients. Maximum number of NI's is known to be caused by Staphylococcus aureus, Pseudomonas aeruginosa, and various gram negative bacilli- E. coli, Klebsiella and Proteus. Similarly the major site of infections are respiratory tract, urinary tract and surgical site. This study was planned to isolate and characterize the bacteria causing NI and to study the sensitivity behaviour of above bacteria. The antimicrobial sensitivity was done by Kirbey and Bauer method. Twenty urine sample and ten swab samples from the surgical site of the local hospital in Haridwar were collected out of which 2 urine and 1 pus sample were positive while remaining are negative. The present study shows that the incidence of occurrence of NI in hospital was found to be 10%.

Keywords: *Nosocomial Infection, Staphylococcus aureus, Pseudomonas aeruginosa, E.coli, Klebsiella, antimicrobial sensitivity.*

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INTRODUCTION:

According to the World Health Organization(WHO) a nosocomial infection(NI) is defined as " An infection occurring in a patient in a hospital or other healthcare facility in whom the infection was not present or incubating at the time of admission"[1].NI are continued to be an important cause of morbidity,mortality, prolonged hospital stay and extra financial burden to patients [2]. Any pathogen present in hospital environment can cause infection because of the compromised immune status of the patients [3]. *Staphylococcus aureus* was perhaps the most important cause of NI that it came to be called "hospital staphylococci". In recent decades the gram negative bacilli- *E.coli, Klebsiella* and *Proteus* have become the most important group of hospital pathogans. *Pseudomonas aeruginosa* has always been important cause of NI because of its intrinsic resistance to antibiotics and ability to survive at low temperature and in disinfection solution.

Common nosocomial infections in patients include surgical site infections,UTI and blood stream infections. Surgical site infections accounts for approx a quarter of all NI. These infections can range from superficial wound infection to necrotizing soft tissue infections [4]. Basa *et al* in their study observed that department of surgery had the highest infection rate. UTI also accounts for a large number of NI in patients. The single most important factor is the indwelling urinary catheter. Having a knowledge of spectrum of organism causing NI and their resistance pattern is important when considering strategies for controlling the development and spread of resistance. Keeping in mind the above facts, this study was planned to delineate the occurrence, microbiology and sensitivity pattern of such infections.

MATERIALS AND METHODS:

Study centre:- This study was incubated on the samples collected from patients admitted to local hospitals of Haridwar during the period of January-March. Twenty urine samples and ten swab samples from surgical site of patients were collected.

Sampling:

1) Air sample :- Exposed plate method was used. Petriplates containing NAM were exposed for 10 minutes and then incubated at 37°C for 24-48 hrs.
2) Urine sample :- Twenty urine samples were collected. Midstream sample of urine is collected aseptically in a sterilized container.
3) Surgical site swab sample :- Ten swab samples are taken. Pus is collected aseptically in an autoclaved container.

Inoculation of bacteria:-

a) From air:-
NAM plates after exposed for 10 min in different wards were incubated at 37°C for 24-48 hrs. Colonies are then counted, purified and maintained for further study.

b) From urine:-
Serial dilution are prepared and marked as 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. 1 ml sample is mixed in distilled water in first test tube known as stock solution. Then further dilutions are prepared by transferring 1ml from stock solution to next test tube and so on. Pipette 1ml from all the dilutions into the sterile petriplate. Then add 15-10ml of NAM, mixed properly and after solidification incubate at 37°C for 24-48 hrs. Only those cases were considered positive in which bacterial count results 10^5 per ml or more.

c) From surgical site swab sample:-
Soaked swabs are streaked in one-fourth of the plate containing medium, then was spread with sterile inoculating loop over the remaining plate. Plates are then incubated at 37°C for 24-48 hrs.

Purification of mixed culture:-
Bacterial population was then grown on selective and differential media like EMB and MacConkey's agar medium by using streak plate method. After obtaining the pure culture, organisms are then gram stained and various biochemical characterizations are done for their identification.

Antibiotic sensitivity test:- Disc diffusion method by Kirby and Bauer(1996) [6] is the technique used. Pasteur Bio discs are used for the accomplishment of the project. First of all, plates are inoculated with the purified organism to be tested for antibiotic sensitivity by spread or streak plate method. Then the Pasteur Bio Discs are placed on the inoculated plates with the help of forcep. After 15 min of application of disc, plates were inverted and placed in the incubator at 37°C for 24hrs. After incubation, plates were examined for a clear zone of inhibition of different diameter was observed around the discs.

Determination of bacterial growth:-In the laboratory, bacterial growth is determined by turbidity measurement method with the help of

photoelectric calorimeter. It involves the inoculation of sterile broth with test organisms and initial OD was taken at 660 nm wavelength, then placed on shaker at 120 rpm and then again OD was taken after a regular interval of time.

RESULTS:

In the present study on "Incidence of common nosocomial infections in hospitalized patients" following results were obtained:-

The air of the hospital consists of *Staphylococcus aureus* (16%), *Micrococcus spp*. (50%) and *Bacillus spp.* (34%)(Table 1).

Table 1:- % occurrence of various bacteria in air of different wards.

LOCATION	BACTERIAL ISOLATE	MEAN NO. OF COLONIES	% OCCURRENCE
Male general ward	***Staph. aureus***	**5**	**10%**
	Micrococcus spp.	**25**	**50%**
	Bacillus spp.	**20**	**40%**
	Total	**50**	
Female general ward	***Staph. aureus***	**30**	**30%**
	Micrococcus spp.	**50**	**50%**
	Bacillus spp.	**20**	**20%**
	Total	**100**	
Private ward	***Micrococcus spp.***	**80**	**50%**
	Bacillus spp.	**80**	**50%**
	Total	**160**	
Emergency (ICU)	***Staph. aureus***	**25**	**25%**
	Micrococcus spp.	**50**	**50%**
	Bacillus spp.	**25**	**25%**
	Total	**100**	

$$\% \text{ occurrence} = \frac{\text{No. of colonies of individual species}}{\text{Total no. of colonies}} \times 100$$

Table 2:- Morphological, cultural and biochemical characterization of isolates.

S. NO.	Characteristics	*Staph. aureus*	*P. aeruginosa*	*E. coli*	*Klebsiella spp.*
A	**Morphological characteristics**				
1	Cell shape	Cocci	Rods	Rods	Rods
2	Cell arrangement	Irregular clusters	Singly	Singly or in pairs	Singl or in pairs
B	**Staining reaction**				
1	Gram staining	Gram +ve	Gram –ve	Gram-ve	Gram-ve
C	**Cultural characteristics**				
1	NAM	Abundant, opaque, golden growth.	Abundant, thin, white mdium turns green.	White, moist, glistening.	Slimy, white, somewhat translucent.
2	MacConky's agar	Bright pink smaller colonies	Forms non lactose fermenting colonies white in colour.	Bright pink, flat colonies.	Bright pink, mucoid colonies
D	**Biochemical characterization.**				
1	Tube catalase	+	+	+	+
2	Gelatin liquefaction	+	+	-	_
	Fermentation:				
3	Lactose	A(+)	-	AG(+)	AG(+)
	Dextrose	A(+)	-	AG(+)	AG(+)
	Sucrose	A(+)	-	A(+)	AG(+)
4	Oxidation/ fermentation test	Fermentative	Oxidative	Fermentative	Fermentative
5	IMViC test:	-	-	+	-
	Indole prod.	+	-	+	-
	MR reaction	+	-	-	-
	VP reaction	-	+	-	+
	Citrate use				
6	Haemolytic activity.	+(β-type)	-	-	-

Table 3:- Zone of clearance (mm) of *Staph. aureus* to antibiotics(average of triplicates).

Antibiotics	Zone of clearance(mm)
Cefotaxime(CF)	19
Pefloxacin(PF)	17
Ofloxacine(OF)	15
Gentamycin(GM)	12
Ciprofloxacin(CP)	11
Ampicillin(AS)	10
Roxythromycin(RF)	1
Co- trimaxazole(BA)	0
Ciphalexin(PR)	0
Tertracycline(TE)	0
Cloxacillin(CX)	0
Lincomycin(LM)	0

Table 4:- Zone of clearance (mm) of *Pseudomona aeruginosa, E. coli and Klebsiella spp.* (average of triplicates).

ANTIBIOTICS	ZONE OF CLEARANCE(mm)		
	E.coli	***P. aeruginosa***	***Klebsiella spp.***
Ampicillin(AS)	8	0	9
Co- trimaxazole(BA)	0	0	0
Cefotaxime(CF)	0	0	0
Piperacillin(PC)	0	10	0
Chloremphenicol(CH)	0	0	5
Ciprofloxacin(RC)	0	20	1
Ceftizoxime(CI)	0	0	2
Tetracycline(TE)	0	0	3
Ofloxacin(ZN)	0	8	0
Amikacin(AK)	0	16	20
Gatifloxacin(GF)	9	0	10
Gentamycin(GM)	7	9	11

Out of the 20 urine samples and 10 surgical site swabs, 2 urine and 1 pus sample were positive cultures. During sampling of human urine and surgical swabs following organisms were isolated :-

Urine*:- Pseudomonas aeruginosa, E. coli and Klebsiella spp.*

Pus:- *Staphlococcus aureus*.

Bacteria were identified on the basis of their morphological, cultural and biochemical characterization (Table 2).

The study of the antibiotics sensitivity against isolated bacteria showed following results:- the zone of clearance of various antibiotics and resistance are as follows:

Staphylococcus aureus (Table 3)

Pseudomonas aeruginosa (Table 4)

E. coli (Table 4)

Klebsiella spp.(Table 4)

Determination of growth:- *Staph. aureus, Klebsiella spp. and Pseudomonas aeruginosa*, all started growing within 6 hrs and reached its maximum at 54 hrs and after that growth started declining. While *E. coli* started growing within 6 hrs and reached its optimum at about 30 hrs and start declining after that.

DISCUSSION:

UTI and surgical site infections are the two most common types of hospital infections. *Staph. aureus* was found to be very common cause of surgical site infections. The explosive outbreaks of *Staph. aureus* infection that were frequently seen in surgical wards demonstrates the infections potential of strain exhibiting particular virulence and colonizing capabilities [7,8].

The ability of *Pseudomonas aeruginosa* to grow in moist conditions with simple nutrients and its

comparative resistance to antibiotics has established it as one of the foremost pathogens [9,10].

In the present study the incidence of UTI in hospitalized patients was found to be 10%. Even with adequate precautions catheterization in hospital leads to urinary infections in 2%, with indwelling cathetars the rate goes to 50% or more(Paniker, 2000).It was also estimated that 5 to 12% of all surgical patients develop post operative infections [11].

During present study the efficacy of different antibiotics was evaluated against *Staph. aureus, E. coli, Pseudomonas aeruginosa and Klebsiella spp*. Fass (1990) [12] observed that ciproflaxin is more active against gram negative disease causing organisms. *E. coli* remains the most common pathogen followed by *Klebsiella pneumonia and Staph. aureus*. Littaua *et al*,1984 [13] ; also reported the similar results. However, Daschner et al and Stratchounski et al [14,15] reported *S. aureus* and *P. aeruginosa* are the most common pathogen in surgical strike infections respectively. Shenoy *et al* [16] also reported that all strains of *P. aeruginosa* in their study were sensitive to imipenem and meropenem. TheAntimicrobial Use Committee in the hospitals will classify antimicrobial agents into the following categories: unrestricted, restricted or reserved, excluded. The antibiotic usage monitoring can be done in pharmacy department and should be reported to the Antimicrobial Use Committee in timely manner. This control programs in turn reduces the antibiotic resistance problems which is a big threat for the hospitals [17].

CONCLUSION:

In the present study it was found that strains isolated showed multiple drug resistance. It concludes that even when hospitalization does not lead to obvious infections it causes change in the patient's microbial flora, the normal flora being gradually replaced by the drug resistance microorganism typically of the hospital environment. So, for the proper management of clinically ill patients and patients undergoing various operative procedures and other medical interventions, hospital antibiotic policies need frequent revisions.

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