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A CLINICAL STUDY OF BACTERIAL PATHOGENS FROM HOSPITAL ACQUIRED INFECTIONS IN ANM MEDICAL COLLEGE AND HOSPITAL, GAYA

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ABSTRACT

Hospital associated infections acquire while receiving health care. It appears 48 hours after Hospital admission or within 30 days after discharge. It is a major problem for patient's safety and has a high impact of poor medical condition and as well as death. The most common infections are Blood, Urinary Tract, Areas of Surgery, Wound infections, Skin infections. The microorganisms which causes these infections are *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Citrobacter* spp., *Enterobacter* spp., *Proteus mirabilis, Acinetobacter baumanii, Staphylococcus aureus, CONS*. The earlier study was based on use of standard specific antibiotic to test the pathogens. In this study, different antibiotics were used to test the sensitivity against these pathogens. The antibiotics were used for the study are Gentamycin, Ciprofloxacin, Amikacin, Ofloxacin, Nitrofurantoin, Norfloxacin, Penicillin, Nalidixic acid, Erythromycin, Amoxillin, Chloramphenicol, Azithromycin, Cefixime, Levofloxacin, Moxifloxacin to test the pathogens. The 398 positive cultures were taken, in which, 216 (54.27%) are urine sample, 46 (11.55%) are Pus sample, 16 are swab sample and 120 surgical site sample. The Antimicrobial Susceptibility was done by Disk Diffusion method (Kirby-Bauer Method). In this study, we have shown that *CONS* (95.2%) has highest antimicrobial susceptibility to Chloramphenicol.

KEY WORDS: Hospital Acquired Infections, Bacterial Pathogens, Antimicrobial Sensitivity, Disk Diffusion Method

INTRODUCTION

Health care-associated infections (HCAIs) are those infections that patients acquire while receiving health care (Horan TC et.al., 2004). Hospital-acquired Infections are those infections acquired in hospital or healthcare service unit that first appear 48 hours or more after hospital admission or within 30 days after discharge following in patient care (Horan TC et. al., 2004). All the different types of infections caused by prolonged stay in hospital accounts for various health issues, often leading to death (Brusaferro S et.al., 2015). It is also called Nosocomial Infections. These include hospitals, housing large number of people who are sick and whose immune systems are often weak. Multiple studies indicate that the most common types of adverse events affecting hospitalized patients are adverse drug events, HCAIs, and surgical complications (Brennan et.al. 1992). Health care-associated Infections is a major problem for patient's safety and has a high impact in terms of morbidity and mortality (Nair et.al., 2017). The most common infections are blood, urinary tract, areas of surgery or pneumonia (Girard R et.al., 2006). Data show, that in countries with high standard of healthcare, 7 out of 100 patients are diagnosed with Nosocomial Infection, but in countries with lower standards of healthcare, the number of infected patients is higher, reaching up to 10 per 100 patients (Khan H.A. et.al., 2017). Nosocomial infections have been classified into 13 types, with 50 infection sites, as according to the National Healthcare Safety Network with Centre for Disease Control (CDC). The common sites of infection are soft tissues, surgical wounds, urinary and respiratory tracts and

intestinal sites (Raka L. et.al., 2006). Wound infections include delayed wound healing or abscess in stitches, other skin infections and cracks due to exogenous pathogens. *Staphylococcus epidermidis*, *Streptococcus pyogenes*, and *Clostridial* species are generally known to cause wound infections. Infection in burns is caused by *Pseudomonas aeruginosa* (Nisreen Husain et.al., 2016). In case of gastrointestinal or urinary tract surgery, wound infections are caused by Gram-negative bacilli, such as *Escherichia coli*. Antibiotic help in superficial healing of wound infections. Deeper organ or tissue infections are cured by surgical drainage and antimicrobial therapy (Mitchell DH.et.al., 1999). UTI is a condition caused by pathogenic invasion of the epithelium, which lines the urinary tract from the minor calyx to prostatic urethra. The Proliferation of Bacteria in the urothelium can be asymptomatic or symptomatic, which causes inflammatory response and symptomatic case characterized by a wide range of symptoms including, fever, lethargy, anorexia and vomiting (Onu GA. et.al., 2013). The pathogens causing such infections are *Escherichia coli*, and the hospital-acquired microbes such as Methicillin-resistant *Staphylococcus aureus* (MRSA) and Antibiotic-resistant *bacilli*. UTI mostly dominated by *E.coli* 75%-80% followed by S. *saprophyticus* 10-15% (Balakrishnan et.al., 2010). And few different species are *Klebsiella*, *Proteus*, *Enterobacter*, *Enterococcus*, *Staphylococcus* and *Pseudomonas aeruginosa*. It can be cured by anti-inflammation therapy (Karzan Mohammed et.al., 2017). Surgical site infection (SSI) is one of postoperative complications in any surgery. Surgical site infections are among most common Nosocomial infections and are encountered in approximately 2%-5% of patients undergoing surgery. These infections are mainly due to hospital acquired infections and irrational use of antibiotics. In developing countries like India, where hospitals have inadequate infrastructure, poor infection control practices, overcrowded wards and practice of irrational use of antimicrobials, the problem of SSIs gets more convoluted (Rajesh Prasad et.al., 2020). Pus is a thick fluid produced as part of the body's response to an infection. Pus is typically an opaque white-yellow color (Macfaddin J et.al., 1976). It's usually odorless, though it may sometimes smell foul. Pus is made up of a buildup of Degenerating white blood cells, Dead/living bacteria, as well as other microorganisms, Tissue debris. They typically are the result of bacteria such as *Streptococcus or Staphylococcus aureus*. The present article emphasizes on the clinical study of some common bacterial pathogens from hospital-acquired infections.

MATERIALS AND METHODS

Sample Collection:

A clinical cross-sectional study (which allows assessment of a disease or a health-related state in a population at a single point in time) was carried in the Department of Microbiology, Anugrah Narayan Magadh Medical College and Hospital, Gaya, Bihar, India for first six months, after taking the approval of the protocol review committee and institutional ethics committee. The Physical parameters of collected specimens were analyzed such as Volume, pH, color, odour and appearance. The different samples (Urine, Pus, Swab, Eye conjunctivitis, Sputum, Surgical site) were collected in Magadh Medical College and Hospital, Gaya District using sterile plastic container, syringe, and cotton swabs aseptically from each patients suspected having Infections in an specific area. Total 501 patients (Male, Female at any age) samples were collected including Urine, Wound, Swab and Pus. The Samples were inoculated in an Nutrient Agar (NA), 5% Sheep Blood Agar (BA) and MacConkey Agar (MA) plates and incubated at 37°C for 24-48 hours before being reported as sterile. Growth on culture plates was identified by its colony characteristics and the standard biochemical tests (Akanmu AO et.al., 2021).

Determination of Antimicrobial Activity:

The susceptibility of the entire isolated organisms to selected antibiotics which were normally used to treat uropathogens was tested by Kirby-Bauer Method. Sterile Mueller-Hinton agar plates were prepared and various antibiotic discs were selected. Identified pathogens were inoculated in peptone water tubes separately and incubated at 37^0 C for 1 hour. Using sterile cotton swabs for each test organism, incubated test organisms were inoculated on the surface of Mueller Hinton agar plates three times, rotating the plate 60^0 after each streaking. Finally the swab was run around the edge of the agar. The cultures were allowed to dry on the plate for 5-10 minutes at room temperature. Various antibiotic discs (Amikacin, Ciprofloxacin, Gentamicin, Nitrofurantoin, Norfloxacin, Cephalaxin, Penicillin, Nalidixic Acid, Cefixime, Levofloxacin, Moxifloxacin, Azithromycin, Ofloxacin,Cefotaxim, Amoxicillin). Were placed on the surface of the agar medium by gently pressing using a sterile forceps on the top of the discs (for better contact and effective diffusion of the antibiotics into the medium). The plates were incubated in an inverted position for 16-18 hours at 37^0 C.

RESULTS AND DISCUSSIONS

Human pathogens were characterized by morphological, microscopical and biochemical tests:

Human pathogens were characterized by morphological, microscopic and biochemical tests. The human Pathogenic bacteria were collected from Magadh Medical College, Gaya and confirmed by its colony morphology, gram staining and Biochemical Tests such as Catalase, Oxidase, Indole, MR, VP, Citrate Utilization Test, Motility Test, Urease Test,

Coagulase Test and Sugar fermentation Test. During the first six month of study period, a total number of 501 specimens, 398 specimens were culture positive (79.45%) (Table 1). Among 398, positive samples 188 (47.24%) were males and positive samples 210 (52.76%) were females. In which 216 (54.2%) samples were urine infection, 120 (30.1%) samples were surgical site infections, 46 (11.56%) were pus infections and 16 (4.0%) were sputum or nasal swab infections. The age wise distribution is shown in (Table 2). There are different organisms that cause Infection. The distribution of number of Human Pathogens causing Infections shown in (Table 3). The Microorganism taken for testing its antimicrobial Susceptibility are: *Acinetobacter baumanii*, *Citrobacter* spp., CONS, *Enterobacter* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*.

Determinant (Gender)	Number (398)	Percentage (100%)
Males	188	47.24%
Females	210	52.76%

Table 1: The number of Gender distribution in Male and Female Patients.

Determinant (Age)	Urine Number	%	Pus Number	%	Swab Number	%	Surgical Site No	%
Below 20	28	(12.9)	4	(8.6)	1	(6.25)	20	(16.6)
20-30	23	(10.6)	5	(10.8)	1	(6.25)	42	(35.0)
30-40	22	(10.1)	7	(15.2)	2	(12.5)	19	(15.8)
40-50	40	(18.5)	6	(13.0)	2	(12.5)	17	(14.1)
50-60	47	(21.7)	8	(17.3)	4	(25.0)	12	(10.0)
Above 60	56	(25.9)	16	(34.7)	6	(37.5)	10	(8.33)
Total	216	(100)	46	(100)	16	(100)	120	(100)

Table 2: The number of Age distribution is determined by the percentage of each infection area in between different ages from below 20 years to above 60 years.

Organism	Total no. of specimens	Urine	%	Pus	%	Swab	%	Surgical Site	%
Acinetobacter baumanii	2	1	(0.46)	-	-	-	-	1	(0.83)
***Citrobacter* spp.**	45	31	(14.3)	1	(2.17)	-	-	13	(10.8)
CONS	21	5	(2.31)	-	-	2	(12.5)	14	(11.6)
***Enterobacter* spp.**	10	3	(1.38)	-	-	-	-	7	(5.83)
Escherichia coli	91	74	(34.2)	3	(6.52)	2	(12.5)	12	(10.0)
Klebsiella pneumonia	82	62	(28.7)	2	(4.34)	-	-	18	(15.0)
Proteus mirabilis	1	1	(0.46)	-	-	-	-	-	-
Pseudomonas aeruginosa	39	22	(10.1)	-	-	-	-	17	(14.1)
Staphylococcus aureus	107	17	(7.87)	40	(86.9)	12	(75.0)	38	(31.6)
Total no. of isolates	398	216	(100)	46	(100)	16	100	120	(100)

Table 3: The number of Organism is determined by the presence of pathogens causes infection in a particular area.

Organism Isolated from Urine, Pus, Swab and Surgical Site Infections					
Antibiotics	***Escherichia coli* Sensitivity %**	***Klebsiella pneumoniae* Sensitivity %**	***Pseudomonas aeruginosa* Sensitivity %**	***Citrobacter* spp. Sensitivity %**	***Enterobacter* spp. Sensitivity %**
	N = 91	**N = 82**	**N = 39**	**N = 45**	**N = 10**
Gentamycin	33 (36.2)	15 (18.2)	11 (28.2)	13 (28.8)	3 (30.0)
Ciprofloxacin	28 (30.7)	26 (31.7)	23 (58.9)	25 (55.5)	5 (50.0)
Amoxicillin	5 (5.49)	7 (8.53)	4 (10.2)	6 (13.3)	1 (10.0)
Ofloxacin	6 (6.59)	3 (3.65)	5 (12.8)	4 (8.88)	1 (10.0)
Nitrofurantoin	29 (27.1)	7 (33.3)	21 (53.8)	31 (68.8)	6 (60.0)
Amikacin	71 (66.3)	14 (66.6)	27 (69.2)	26 (57.7)	5 (50.0)
Norfloxacin	62 (57.9)	12 (57.1)	1 (2.56)	1 (2.22)	1 (10.0)
Erythromycin	64 (59.8)	13 (61.9)	29 (74.3)	37 (81.1)	7 (70.0)
Chloramphenicol	54 (50.4)	10 (47.6)	11 (28.2)	14 (31.1)	2 (20.0)
Azithromycin	78 (72.8)	15 (71.4)	3 (7.69)	2 (4.44)	1 (10.0)
Levofloxacin	76 (71.0)	13 (61.9)	31 (79.4)	36 (80.0)	8 (80.0)
Cefixime	15 (16.4)	17 (20.7)	14 (35.8)	15 (33.3)	3 (30.0)
Moxifloxacin	51 (56.0)	53 (64.6)	26 (66.6)	24 (53.3)	4 (40.0)
Cephalexin	6 (6.59)	8 (9.75)	2 (5.12)	5 (11.1)	1 (10.0)
Cefotaxime	11 (12.0)	16 (19.5)	13 (33.3)	10 (22.2)	2 (20.0)

Table 4: The Antimicrobial Sensitivity is determined by percentage measures from sensitivity of all different antibiotics (15) against Gram –ve Bacteria.

Organism Isolated from Urine, Pus, Swab and Surgical Site Infections		
Antibiotics	***Staphylococcus aureus* Sensitivity %**	**CONS Sensitivity %**
	N = 107	**N = 21**
Gentamycin	87 (81.3)	17 (80.9)
Ciprofloxacin	89 (83.1)	18 (85.7)
Amikacin	34 (31.7)	9 (42.8)
Ofloxacin	29 (27.1)	6 (28.5)
Norfloxacin	46 (42.9)	11 (52.3)
Erythromycin	39 (36.4)	8 (38.0)
Amoxicillin	47 (43.9)	12 (57.1)
Chloramphenicol	97 (90.6)	20 (95.2)
Azithromycin	82 (76.6)	15 (71.4)
Levofloxacin	49 (45.7)	13 (61.9)

Table 5: The Antimicrobial Sensitivity is determined by percentage measures from sensitivity of all different antibiotics (15) against Gram +ve Bacteria.

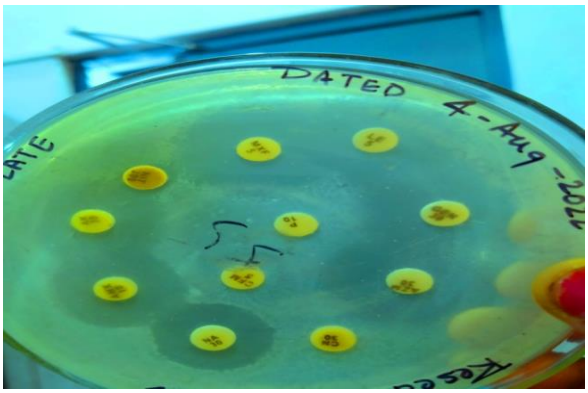

Figure 1: The Antibiotics Nalidixic acid, Amoxicillin, Azithromycin, Moxifloxacin, Gentamycin, Levofloxacin, Amikacin, gives clear zone of Inhibition against *E.coli* in MHA Plate.

Antibiotics showed antimicrobial activity against the tested human pathogens:

The age wise distribution of the gender has been shown in the (Table 2) with maximum no. of culture positive samples in above 60 years (25.9%), followed by 50-60 (21.7%) and then followed by 40-50 (18.07%) of age group respectively. Out of 398 culture positive samples *Staphylococcus aureus* (26.88%) was the most common pathogen isolated followed by *Escherichia coli*. (22.86%), *klebsiella* spp. (20.60%) and *Citrobacter* spp. (11.30%) respectively (Table 3). Among gram negative *Bacilli, E.coli* was most sensitive to Azithromycin (72.8%), followed by Levofloxacin (71.0%) where as Amikacin (66.3%), Erythromycin (59.8%), Norfloxacin (57.9%) and Moxifloxacin (56.0%) was the drug of choice. (Table 4) And for *Klebsiella* spp. was most sensitive to Azithromycin (71.4%) where as Amikacin (66.6%), Moxifloxacin (64.6%) and Norfloxacin (57.1%) was the drug of choice. (Table 4). For *Pseudomonas aeruginosa* Erythromycin (81.1%) showed maximum sensitivity followed by Levofloxacin (80.0%) where as Ofloxacin (68.8%), Ciprofloxacin (55.5%) and Moxifloxacin (53.3%) was the drug of choice. (Table 4). Among Gram Positive *Cocci Staphylococcus aureus* was the most sensitive to Chloramphenicol (90.6%) followed by Ciprofloxacin (83.1%), Gentamycin (81.3%). Whereas *CONS* was most sensitive to Chloramphenicol (95.2%), followed by Ciprofloxacin (85.7%), Gentamycin (80.9%). The hospital-associated infections appeared long before the origination of hospitals. This spread fast as the health problem during antibiotic era. These infections led to the increase in the cost of the antibiotics and their use as well, with an extended hospitalization. This elevated the incidences of morbidity and mortality. In the Present Study, Staphylococcus aureus (26.8 %) ranked highest in occurrence compared with other bacterial isolates encountered. This finding agrees 9 with the study conducted by Gadzama et al. In all clinical samples, E. coli showed high resistance rates of > 80% to erythromycin (Bharathi MJ et.al. 2002). Gentamycin (81.3%), Ciprofloxacin (83.1%), and Chloramphenicol (90.6%), Shows maximum susceptibility. The antibiogram profile of staphylococcus aureus showed that most of the strains encountered were mostly susceptible to Ciprofloxacin (87 %) (Wariso BA et.al. 2006). E. coli isolates were sensitive to Gentamicin, Nitrofurantoin, Ciprofloxacin and Chloramphenicol. Similar studies conducted in Ethiopia (Forbes BA et.al. 1998) and Nigeria (Akanmu AO et.al. 2021) have reported comparable susceptibility rates. High sensitivity to ciprofloxacin and Gentamicin and Norfloxacin has been recorded from previous studies conducted in Nigeria and India (Forbes BA et.al. 1998). In this study, Norfloxacin, Ciprofloxacin, Gentamicin and Chloramphenicol were found to be the most effective antimicrobials against E. coli isolates.

CONCLUSION

The results of this study show high rates of antimicrobial resistance to erythromycin, Amikacin. Nitrofurantoin, Norfloxacin Gentamicin, ciprofloxacin, Levofloxacin, Ofloxacin, Azithromycin are considered appropriate for empirical treatment of all Human Pathogens in the study area. Periodic monitoring of antimicrobial susceptibility both in the community and hospital settings is recommended.

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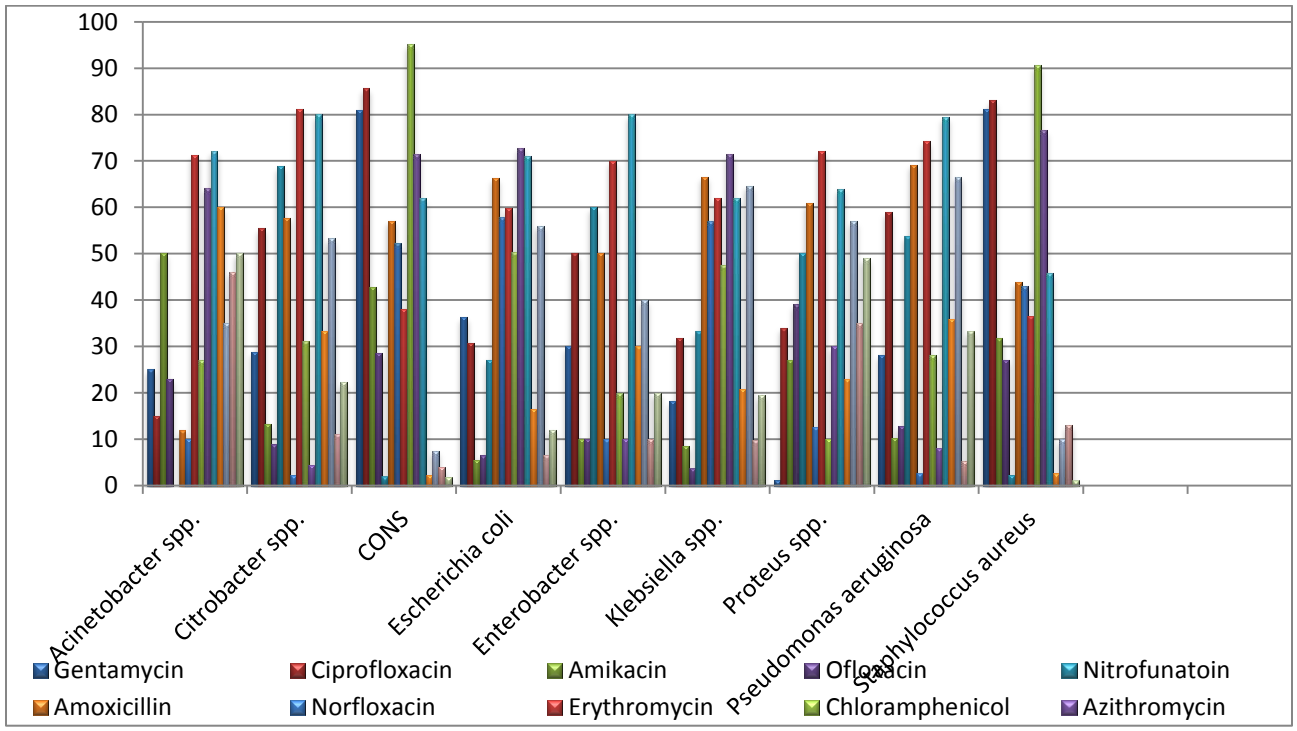

Figure 2: Graph showing antimicrobial sensitivity, expressed as zone of inhibition, pathogens. The higher pick in the graph shows the susceptibility rate of Chloramphenicol (95.2%) against Human Pathogens.

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