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RESEARCH ARTICLE

A BACTERIOLOGICAL STUDY OF NOSOCOMIAL INFECTIONS IN AN INTENSIVE CARE UNIT IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Nosocomial infections are applied to infections developing in hospitalized patients, not present or without incubation at the time of their admission. The present study was undertaken to determine most predominant bacterial isolates in nosocomial infections of ICU, their antibiotic sensitivity pattern and to identify the specific age group of people at a higher risk of acquiring nosocomial infections in ICU. 100 samples were studied over a period of 3 months in a tertiary care hospital. Clinical specimens collected from patients admitted in ICU at least for 48 hours with suspicion of nosocomial infections were included. Isolates were identified by standard procedures and antibiotic sensitivity done by Kirby Bauer method. 38(38%) of the clinical specimens were culture positive while 62 specimens showed no growth. Male to Female ratio was 2.03:1. Of 40 isolates recovered, 28(70%) were Gram negative bacilli, eight (20%) were Gram positive cocci, one (2.5%) Candida spp., three (7.5%) were non-fermenters. The most common organisms isolated was *Klebsiella pneumoniae* (42.5%) followed by Methicillin Resistant *Staphylococcus aureus* (15%). All isolates of *Klebsiella pneumoniae* were sensitive to Imipenem and Piperacillin-Tazobactam acid. Nosocomial infections was found to be more prevalent in young adults (21 to 30 years). This study clearly documents a high prevalence rate of nosocomial infections in the ICU, Gram-negative bacteria being the most common causative pathogens. There is an alarmingly high rate of resistance to cephalosporins and β lactam-β-lactamase inhibitors among Gram negative organisms. Judicious use of older and newer antimicrobial agents is essential to prevent the emergence of multi drug resistant bacteria in the ICU. We recommend that education and awareness among health care workers as well as adherence to standard guidelines for prevention of nosocomial infections.

INTRODUCTION

Nosocomial infections are applied to infections developing in hospitalized patients, not present or without incubation at the time of their admission. Such Infections may become evident during their stay in hospital or sometimes only after their discharge. Nosocomial infections are typically exogenous, the source being any part of the hospital ecosystem, including people, objects, food, water and air. Such infections may be iatrogenic in that they may be induced by some diagnostic or therapeutic intervention in the hospital. They may be opportunistic in that microorganisms of low virulence may cause disease in hospitalized patients whose immune mechanisms are impaired. Approximately 5-10 per cent of patients admitted to acute care hospitals in developed countries and more than 25 per cent of such patients from developing countries have been found to acquire Nosocomial infections. Such Nosocomial infections add to the morbidity, mortality, and costs that one might expect from the underlying illness alone. This is tragic since it is believed that as many as 20 per cent of Nosocomial infections in developed countries and 40 per cent in developing countries are preventable (Ananthanarayan *et al*., 2009). ICUs have become increasingly important in the past 20 years as the location where many of these infections occur. ICU acquired infections are 5-10 times more than in general ward patients (Richards *et al*., 2003). Nosocomial infections are frequently encountered in ICUs because of the severity of underlying diseases, the frequency of invasive interventions, and the frequent use of wide-spectrum antibiotics. It has been reported that ICUs account for 25% of nosocomial infections, even though they occupy only approximately 10% of bed capacity of the hospital (Ramana and Chaudhury, 2012). Also the situation is further complicated by emergence of multi drug resistant pathogens. Critically ill patients are always at higher risk of developing nosocomial infections with resistant strains (Goel *et al*., 2009). In a recent report, Infectious Disease Society of America specifically addressed 3 categories of Gram Negative Bacilli (GNB) namely Extended Spectrum B-Lactamase (ESBL) producing Escherichia Coli and Klebsiella, Multi Drug Resistant (MDR) Pseudomonas and carbapenem resistant acinetobacter species as high priority bacterial pathogens

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(Talbot *et al*., 2006). Antimicrobial resistance in nosocomial infections is increasing with both morbidity and mortality greater when infection is caused by drug resistant organisms. This increase is due to overuse and misuse of antimicrobial agents, immunosuppressed patients and exogenous transmission of bacteria, usually by hospital personnel. Nosocomial infections are typically exogenous, the source being any part of the hospital ecosystem, including people, objects, food, water and air in the hospital. These infections are opportunistic and microorganisms of low virulence can cause disease in hospital patients whose immune mechanisms are impaired. The outcome is that many antibiotics can no longer be used for the treatment of infections caused by such organisms and the threat to the usage of other drugs increases (Patwardhan *et al*., 2008).

All these major reports indicate the need for obtaining data on prevalent strains in the ICU along with the susceptibility pattern, to help in revising antibiotic policy and guiding clinicians for the better management of patients. Prevalent flora and antimicrobial resistance pattern may vary from region to region depending upon the antibiotic pressure in that locality (Goel *et al*., 2009). Surveillance of nosocomial infections helps in determining infection rates, risk factors and in further planning the preventive strategies to ensure a quality health care in any hospital (Ramana and Chaudhury, 2012). The increased prevalence of resistant organism in ICU indicates the need to strengthen the antibiotic policy thereby avoiding the prolonged and indiscriminate use of antimicrobial agent. The present study is to precisely define the interrelationships between underlying disease, severity of illness, therapeutic activity, and nosocomial infections in ICU patients, and their respective influences on these patients' outcome. In present study we attempted to determine the predominant bacterial isolates from hospital acquired infection of intensive care units and their susceptibility pattern to the anti microbial agents currently used in the treatment of infections. We have also attempted to know the specific age group at the higher risk of acquiring Nosocomial Infections.

Aims & Objectives

- To know most predominant bacterial isolates in Nosocomial Infections of ICU.
- Antibiotic sensitivity pattern of bacterial isolates within the predominant ones of ICU.
- To identify the specific age group of people at a higher risk of acquiring Nosocomial Infections in ICU.

MATERIALS AND METHODS

After obtaining clearance from Institutional Ethics Committee (IEC), this study was is done in the Department of Microbiology of a tertiary care teaching hospital attached to a Medical College. We studied 100 samples were over a period of 3 & half months (June 15th 2012 to September 31st 2012). Specimens included are endotracheal secretions, throat swab, sputum samples, blood, urine, peritoneal fluid and pus, which are collected aseptically from patients of all age groups.

Inclusion and Exclusion criteria:- Patients admitted in ICU at least for 48 hours are included. Isolates from repeat culture of previously recruited patients and isolates identified as commensals or contaminants are excluded. All specimens are routinely cultured on MacConkey and blood agar plates. Blood specimens are inoculated in Brain heart infusion broth and subcultured on chocolate agar on alternate days for 7 days. All suspected colonies are identified by gram-staining, colonial morphology and other biochemical reactions according to standard procedures (Forbes *et al*., 2007). Antimicrobial susceptibility testing of the isolated organisms is performed by disk diffusion method as recommended by CLSI guidelines as follows.

The Kirby Bauer Disc Diffusion method

This is done on Muller Hinton agar (Hi Media, Mumbai) which is prepared from a dehydrated base according to the manufacturer's instructions. The preparation of the inoculum for the sensitivity testing is done by emulsifying 3-5 morphologically similar colonies in peptone broth and incubating them at 37^{o}c until turbidity is comparable to a 0.5 Mc Farland's Turbidity standard. The control is prepared by using the E coli ATCC 25922 strain. A sterile cotton swab is dipped into the inoculum and rotated several times against the wall of the test tube above the fluid level, to remove the excess inoculum. The dried surface of Muller Hinton plate is then inoculated with the swab as a Lawn culture. Once the surface is dried, the antibiotic discs (from Hi Media, Mumbai) are placed on the surface by evenly spacing them in such a way that any two discs are not closer than 24mm from centre to centre. After 18 hrs of incubation at 37^{o}c, the inner diameter of zone of inhibition is measured by using a millimetre scale around each antimicrobial disc, on the undersurface of the plate. The zone size around each antimicrobial disk is interpreted as sensitive, intermediate or resistant according to the CLSI guidelines of 2011 (CLSI, 2011).

The antibiotics which are tested for Gram negative organisms include Amikacin (10µg), Amoxiclav (Ampicillin-Sulbactam (10/10µg), Aztreonam (30µg), Cefuroxime (30µg), Ceftazidime (30µg), Ceftazidime-Clavulanic acid (30/10µg), Cefepime (30µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Cotrimoxazole (1.25/23.75µg), Gentamycin (10µg), Imipenem (10µg), Piperacillin-Tazobactam (100/10µg). sThe antibiotics which are tested for Gram positive organisms include Amoxiclav (Ampicillin-Sulbactam (10/10µg), Cefoxitin (30µg), Cefuroxime (30µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Cotrimoxazole (1.25/23.75µg), Gentamycin (10µg), Clindamycin (2µg), Linezolid (30µg), Oxacillin (1µg), Penicillin G(10units), Tetracyclin (30µg), Vancomycin (30µg). All antibiotic disks are obtained from Himedia ltd (Mumbai, India).

RESULTS

During the study period, laboratory data of 100 patients whose specimens were received was evaluated. Male to Female ratio was 2.03:1. Specimens were Endotracheal secretions-35, urine-22, blood-19, sputum-17, pus-5, peritoneal fluid-2. Out of 100 specimens, 38(38%) were culture positive while 62 specimens showed no growth. Culture positive specimens were Endotracheal secretions-19, sputum-12, urine-3, pus-3, blood-1. From 38 culture positive specimens, 40 isolates were recovered. In an ET and pus specimens, there were two isolates

Table 1. Different microorganisms isolated from the specimens of intensive care unit patients

Organisms	Endotracheal secretions	Sputum	Pus	Urine	Blood	Total
Klebsiella pneumoniae	09	05	03	00	00	17(42.5%)
MRSA	03	00	00	02	01	06(15%)
Klebsiella oxytoca	03	00	00	00	00	03(7.5%)
Pseudomonas aeruginosa	01	00	00	00	00	01(2.5%)
Escherichia coli	02	00	00	00	00	02(5%)
Enterobacter	00	01	00	00	00	01(2.5%)
Citrobacter	00	01	00	00	00	01(2.5%)
Candida albicans	00	00	00	01	00	01(2.5%)
Coagulase negative Staph	00	02	00	00	00	02(5%)
Gram negative non-fermenters	02	00	01	00	00	03(7.5%)
Non-commensals	00	03	00	00	00	03(7.5%)
Total	20	12	04	03	01	40(100%)

Table 2. Antimicrobial sensitivity pattern for the gram negative bacilli recovered from Intensive Care Unit patients

Antibiotics	Klebsiella pneu (17)	Klebsiella oxytoca (03)	Gram negative non-ferm (03)	Pseudomonas aeruginosa (01)	Escherichia coli(02)	Entero-bacter (01)	Citro-bacter (01)
Amikacin	10(59%)	01(33%)	02(66%)	NO	02(100%)	01(100%)	NO
Amoxiclav	05(29.4%)	03(100%)	NO	01(100%)	01(50%)	01(100%)	NO
Aztreonam	04(23.5%)	NO	NO	NO	NO	01(100%)	NO
Cefuroxime	02(11.8%)	NO	NO	NO	NO	NO	NO
Ceftazidime	02(11.8%)	NO	NO	01(100%)	NO	01(100%)	NO
Ceftazidime-clavilunate	02(11.8%)	NO	NO	01(100%)	NO	01(100%)	NO
Cefepime	04(23.5%)	01(33%)	NO	01(100%)	NO	01(100%)	NO
Chloramphe-nicol	06(35.3%)	01(33%)	NO	01(100%)	01(50%)	NO	NO
Cipro-floxacin	04(23.5%)	01(33%)	01(33%)	NO	01(50%)	NO	NO
Cotri-moxazole	04(23.5%)	NO	NO	01(100%)	NO	NO	NO
Gentamicin	05(29.4%)	NO	01(33%)	01(100%)	NO	NO	NO
Imipenem	17(100%)	03()	03()	01(100%)	02(100%)	01(100%)	1(100%)
Piperacillin-Tazobactam	17(100%)	03()	03()	01(100%)	02(100%)	01(100%)	1(100%)

Table 3. Antimicrobial sensitivity pattern for the gram positive cocci from Intensive Care Unit patients

Antibiotics	MRSA(06)	Coagulase negative staph(02)
Amoxiclav	NO	NO
Cefoxitin	NO	NO
Cefuroxime	NO	NO
Chloramphenicol	NO	NO
Ciprofloxacin	03(50%)	01(50%)
Cotrimoxazole	NO	NO
Gentamicin	02(33.33%)	NO
Clindamycin	02(33.33%)	NO
Linezolid	06(100%)	02(100%)
Oxacillin	NO	NO
Penicillin G	NO	NO
Tetracyclin	04(66.66%)	01(50%)
Vancomycin	06(100%)	02(100%)

per specimen (5.3%) and 36 specimens showed growth of single organism. Out of 40 isolates, 28(70%) were GNB, eight (20%) were GPC, one (2.5%) Candida spp., three (7.5%) were non-commensals.

Table 1 represents the distribution of various organisms recovered from various specimens of ICU patients. The common organisms in decreasing order of frequency are Klebsiella pneumoniae-17(42.5%), MRSA-six (15%), Gram negative non-fermenters -three (7.5%), *Klebsiella oxytoca*-three (7.5%).

Table 2 represents Antimicrobial sensitivity pattern for the gram negative bacilli recovered from Intensive Care Unit patients. Imipenem (100%) and Piperacillin-Tazobactam (100%) were the most effective in vitro drugs against all gram negative bacilli followed by Amikacin, Amoxiclav and Chloramphenicol. *Klebsiella pneumoniae* was least sensitive to Cefuroxime (11.8%), Ceftazidime (11.8%) and Ceftazidime-Clavulanic acid (11.8%).

Table 3 represents Antimicrobial sensitivity pattern for the gram positive cocci from Intensive Care Unit patients. Vancomycin 100%) and Linezolid (100%) were the most sensitive drugs in vitro followed by Ciprofloxacin (50%) which was sensitive to some MRSA isolates.

Table 4. Prevalence of Nosocomial Infections among various age groups in an Intensive Care Unit

Age Group	Males		Females		Total		
	Tested	Culture positive	Tested	Culture positive	Tested	Culture positive	Percentage
15-20 yrs	03	01	08	03	11	4	10%
21-30yrs	20	12	03	01	23	13	32.5%
31-40 yrs	04	00	07	02	11	2	5%
41-50yrs	18	06	06	02	24	8	20%
51-60yrs	16	06	03	01	19	7	17.5%
61-70yrs	06	03	06	03	12	6	15%

Table 4 represents Prevalence of Nosocomial Infections among various age groups in an Intensive Care Unit. Highest prevalence was seen in young adults of age range 21 to 30(32.5%); followed by middle aged 41 to 50 years having 20% prevalence and 51 to 60 years each having 17.5% prevalence.

Table 5. Predominant isolates from ICU Nosocomial infections as reported by various recent studies

Organism	Present study 2012	Ramana BV 2012	Sood 2011	Goel N 2009	Patwardhan 2008	Agarwal 2005
Klebsiella pneumoniae	42.5%	18%	10.52%	13.6%	19.11%	-
Pseudomonas aeruginosa	2.5%	33.3%	10.52%	35%	13.24%	23.9%
Acinetobacter	-	-	26.31%	23.6%	13.24%	34.8%
Escherichia coli	5%	16%	21.05%	7.4%	27.2%	15.2%
MRSA	15%	7.6%	-	1.8%	17.28%	-
Gram Negative non fermenters	7.5%	12.1%	-	-	-	-

Table 6. Sensitivity pattern of Klebsiella pneumoniae as reported by various studies

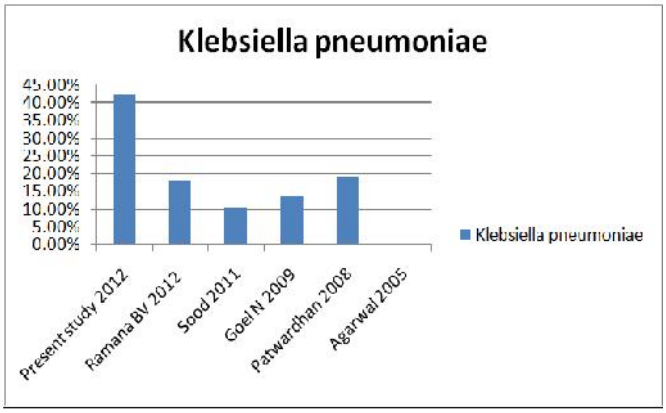
Antibiotics	Present study 2012	Ramana BV 2012	Goel N 2009	Patwardhan 2008
Amikacin	58.82%	54.8%	26.4%	61.1%
Amoxiclav	29.41%	-	NO	-
Aztreonam	23.5%	-	-	-
Cefuroxime	11.8%	-	-	30%
Ceftazidime	11.8%	-	5%	11.1%
Ceftazidime-Clavulanic	11.8%	-	-	-
Cefepime	23.5%	-	-	-
Chloramphenicol	35.3%	-	-	22.2%
Ciprofloxacin	23.5%	35.8%	4.5%	5.5%
Cotrimoxazole	23.5%	-	9.1%	-
Gentamicin	29.41%	35.8%	-	11.1%
Imipenem	100%	74%	91%	-
Piperacillin-Tazobactam	100%	81%	-	-

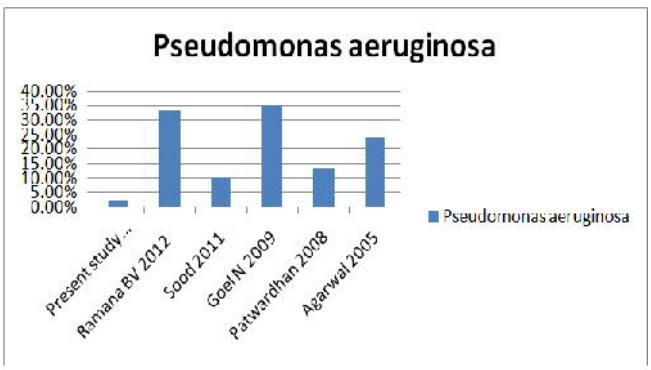
DISCUSSION

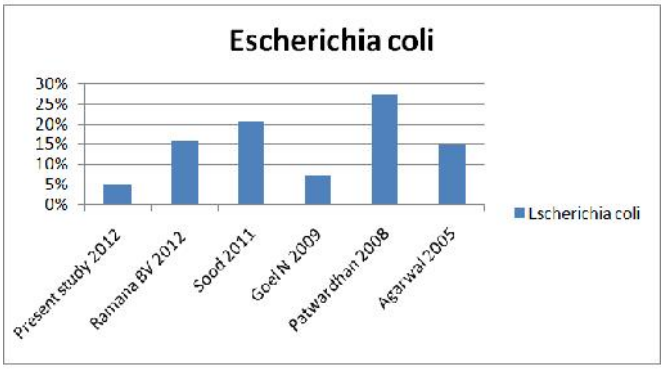
Nosocomial infections are becoming an increasing problem for hospitalized patients; especially in the ICU is a serious cause of concern for hospitals (Singh *et al*., 2010). The risk factors for Nosocomial infections include; diabetes mellitus, intubation, persistant sounding, surgical drains, poor health status, lack of using gloves, irregular and inappropriate debridement and wound bandage (Agarwal and Thomas, 2003). The prevalence of Nosocomial infections in critical care unit is high. In addition they are impose heavy cost on hospitals, causing increased hospitalization time, increased morbidity and mortality (Rahim baghaei *et al*., 2011). The infections are contagious and may increase the mortality and morbidity in other patients (Alberti *et al*., 2002). Antimicrobial resistance in Nosocomial infections is increasing with both morbidity and mortality greater when infections is caused by drug resistance organism (Hosein *et al*., 2002). The outcome is that many antibiotics can no longer be used for the treatment of infections caused by such organisms and the threat to the usage of other drugs increases (Courvalin, 1996; Chopra, 1998).

Table 5 & Figure 1 shows Predominant isolates from ICU Nosocomial infections as reported by various recent studies being compared with our study findings. Klebsiella pneumoniae is the commonest organism in our study in correlation to Goel *et al*. (2009), Ramana *et al*. (2012), Patwardhan *et al*. (2008) and Sood *et al*. (2011) who had also isolated *Klebsiella pneumoniae* as one of the predominant ICU pathogen. Other common pathogens in our study were MRSA, Klebsiella oxytoca and Gram Negative Non-Fermenters in contrast to Ramana *et al*. (2012) with *Pseudomonas aeruginosa* and *Escherichia coli*, Sood *et al*. (2011) with *Acinetobacter spp*. and *Escherichia coli*, Goel *et al*. (2009) and Agarwal *et al*. (2005) with *Pseudomonas aeruginosa* and Acinetobacter while Patwardhan *et al*. (2008) isolated *Escherichia coli* in addition to MRSA. In 5.3% cases, two isolates were recovered from a single specimen, in contrast to the other study that reported two to three isolates per specimen in 10.75% cases (Goel *et al*., 2009).

Table 6 shows Sensitivity pattern of Klebsiella pneumoniae as reported by various studies being compared with the present study. Predominant Gram negative organisms Klebsiella pneumonia and others GNB totally showed least sensitivity to Cefuroxime followed by Aztreonam, Ceftazidime, Ceftazidime-clavulanic acid, Cotrimaxozole. High rate of resistance at our center might be due to the selective influence of extensive usage of third generation cephalosporins. Nearly all isolates were sensitive to Imipenem supporting the fact that Carbapenems can still be used as a last choice in treating serious infections caused by GNB.







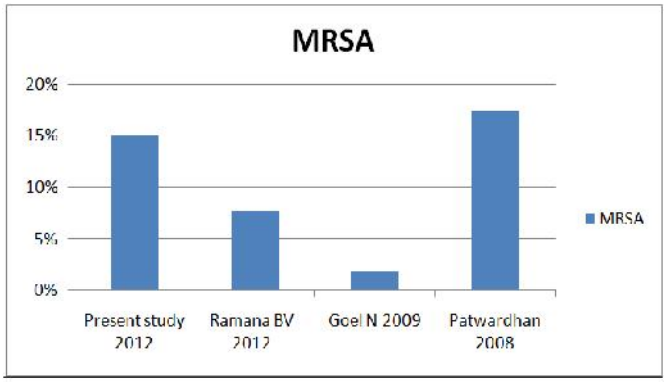


Figure 1. Comparison of prevalence of bacterial isolates in various studies

None of the isolates of *Klebsiella* spp., were resistant to Imipenem in contrast to another study, where resistance to Carbepenem group drugs was found in 9% of isolates (Goel *et al*., 2009). Commonest age group which acquire nosocomial infections was found to be 21 to 30 years because of increased number of ICU admissions due to OP poisoning, Road Traffic Accidents (RTA) in that age group.

This study clearly documents a high prevalence rate of nosocomial infections in the ICU, Gram-negative bacteria being the most common causative pathogens. There is an alarmingly high rate of resistance to cephalosporins and β lactam-β-lactamase inhibitors among Gram negative organisms. Imipenem (100%) and Piperacillin-Tazobactam (100%) were the most effective in vitro drugs against all GNB. Vancomycin (100%) and Linezolid (100%) were the most sensitive drugs against GPC. Judicious use of older and newer antimicrobial agents is essential to prevent the emergence of multi drug resistant bacteria in the ICU. Nosocomial infections are found to be more prevalent in young adults due to more number of ICU admissions. Prevention is more important than management of Nosocomial infections, simple measures of asepsis while insertion of catheters and general management of hygiene can decrease the incidence of nosocomial infections. We recommended that education and awareness among health care workers as well as adherence to standard guidelines for prevention of nosocomial infections.

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