

Activities of Antibiotics against Drug-Resistant Clinical Nosocomial Pathogens

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Multiple surveillance studies have demonstrated that resistance among prevalent pathogens is increasing at an alarming rate, leading to greater patient morbidity and mortality from nosocomial infections. Among 50 patients the isolated nosocomial pathogens are *Staphylococcus sp*, *Streptococcus sp*, *Pseudomonas sp*, *E.coli*, *Klebsiella sp*, *Enterobacter sp* and *Bacillus sp*. Of these organisms *Staphylococcus sp* (35%) and *Klebsiella sp* (30%) are the dominant microbial pathogens. The drug resistance and sensitivity pattern of nosocomial bacterial pathogens were tested against antibiotics such as Ampicilin, Amoxycilin, Cefazolin, Gentamicin, Neomycin, Rifampicin, Tetracycline, and Vancomycin. In *Staphylococcus sp* shows highly resistant against Gentamicin (19mm) and Neomycin (24mm) was highly controlling and sensitivity for Rifampicin (6mm) and Vancomycin (8mm) but susceptible for Amoxycilin, Cefazolin and Tetracycline. *Klebsiella* show highly resistant against Cefazolin (18mm) and Neomycin (24mm) was highly controlling and sensitivity for Amoxycilin (6mm) and Vancomycin (2mm) but susceptible for Rifampicin and Tetracycline. Appropriate antibiotic selection, surveillance systems, and effective infection-control procedures are key partners in limiting antibiotic-resistant pathogen occurrence and spread.

Key words: Antibiotics, Nosocomial, Pathogen, *Staphylococcus sp*, *Klebsiella sp*.

Nosocomial infections are defined as infections occurring in patients after admission to hospital. Not only patients but even staff members of hospitals who have contact with patients can suffer from nosocomial infections. It was reported that around 5% of patients who were hospitalized acquired infection in the hospital (Sainanli, 1999). Much more commonly, patients acquired hospital infections with common organisms because of their own great susceptibility to infection or because of procedures carried out in the hospital, (Werdegard *et al.*, 1985). Some examples of inanimate exogenous sources are food, urinary catheters, intravenous

and respiratory therapy equipment and water systems (Prescott, 1980).

The world health organization (WHO) has recognized the serious global problem of nosocomial infections. Mortality associated with nosocomial infections also difficult to determine, but in united states, it was estimated that of 2,00,000 deaths in patients with nosocomial infections, 20,000 were attributable directly to the infections and further 60,000 it contributed to death. The cost of nosocomial infections was approximately 120 million per year in England and Wales in 1987 based on an average additional stay of days for each hospital acquire infections (Crook and bowler, 1982). In the hospital, surveillance is utilized to determine endemic or back ground rates of infection, to identify high – risk locations and procedure and to detect a significant rise in the rate of infection above endemic rates, indicating

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an epidemic that requires immediate investigation. More commonly, surveillance is used to set priorities for infection control activities (Farber and Wenzel 1996). Nosocomial infection was well protected from colonization by microorganisms but this nosocomial region has no resident flora but may transiently contaminate by inhaled organisms (Nester *et al.*, 2000).

India has shown the β - Streptococci and Staphylococcus pneumoniae are the most predominant bacteria isolated from nosocomial infection cases. Risk factors like low birth weight, malnutrition and poor sanitation influence the incident and severity of nosocomial infection in India. *Pseudomonas* sp. is a Gram negative motile microorganism, which was responsible to cause nosocomial infection (Aka *et al.*, 1997). An outbreak of gastro enteritis due to vibrio parahaemolyticus (Stephen and Lalitha 1993) studied an outbreak of Serratia marcescens infections, among obstetric patients (Lalitha *et al.*, 1983). A comparative evaluation of two indigenously developed tests for rapid detection of group A Streptococci directly from throat swabs (Rita & Awdhesh 1997). A combination of selective surveillance methods for hospital acquired infections in a 350 bed hospital over a period of 7 years (Anandita and Bibhabati 2000). In investigated the effect of fumigation on bacteriology of operation theatres (Shubhangi kamat *et al.*, 1997).

Introduced urinary tract Nosocomial Infection in genetric and institutionalized patients (Nicolle, L.B 2002). Studied the prevalence of Nosocomial Infections in 15 Italian hospitals (Nicostrì E, Petrassilla N, and Martini L *et al.*, 2003). Studied the hospital acquired infection surveillance in a neurological Intensive Care Unit (Orsi G.B 2006). Studied device associated Nosocomial infection in 55 Intensive Care Unit of 8 developing countries (Rosenthal V.D, Maki D.G 2006). Studied the epidemiology, risk factor and outcome if Nosocomial Infection in a Respiratory Intensive Care Unit in North India reported that the incidence of Nosocomial viral respiratory infection in neonates (Agarwal R, Gupta D, Rey P, Aggarwal A.N, Jindal S.K 2007). Gagner A (2007). The fungi are common pathogens of Nosocomial infection in very low birth weight infants (Huang 2008). The Nosocomial infection is a major risk for premature with very low birth (Basker C.J, Malish M 2008). A case studied the Hand contamination before after different hand hygiene techniques (Lucel J.C, Rigand M.P 2008).

MATERIALS AND METHODS

Collection of nosocomial samples

Preparation of Throat Swab samples

Sterile tongue depressor was used to approach the swabbing area. A throat swab specimen was collected properly in order to avoid contamination with the saliva. A sterile cotton wool swab was placed in a sterile tube for the collection of the specimen. The tongue was depressed and two swabs were passed well over the tonsils and tonsillar fossil and also over the posterior pharyngeal wall. From those two swabs, one was used for smear preparation and other was used for culturing the nosocomial microbial pathogens. After collecting the samples, the swabs were introduced into 10ml of sterile Brain Heart Infusion broth. The inoculated BHI broths were placed in the ice box and transported to the laboratory immediately for further analysis.

Enumeration of the Nosocomial Pathogens

Plating Procedure

The inoculated BHI broths were incubated in a bacteriological incubator at 37°C for 24 h to allow the growth of nosocomial pathogens. The specific medium such as Blood Agar, Mac Conkey Agar, Mannitol salt agar, Eosin Methylene Blue Agar (EMB) and Mannitol salt agar, medium were poured into Petri plates. The plates were allowed to solidify. After solidification one loopful of sample from BHI broth were streaked on Blood agar, Mac conkey agar, EMB agar and Mannitol salt agar individually. The blood agar, Mac Conkey agar plates, EMB agar plate and Mannitol salt agar plates were incubated in bacteriological incubator at 37°C for 24 h.

Identification and biochemical test for the nosocomial pathogens isolated

Morphological analysis

Gram staining:

Gram staining technique was used to separate bacteria into two groups such as, Gram positive and Gram negative. Bacterial smear was prepared in a clean glass slide. The smear was flooded with crystal violet and allowed it for 1 minute. The excess stain was washed in tap water gently. Then the smear was flooded with gram's iodine for 1 minute and washed. Then 95 % ethyl alcohol was added over slide drop wise for 20 to 30 seconds and the slide was washed under tap water.

Then the slide was flooded with counter stain saffranin for 1 to 2 minutes, and was washed with tap. Slide was examined using microscope under oil immersion objective lens.

Motility

The hanging drop technique was followed to observe the motility of the organisms. The petroleum jelly was applied at the four corners of the cover slip with the help of applicator stick. One loop full of overnight culture was placed on the centre of the cover slip, with sterile inoculation loop and inverted the cavity glass slide. The slide was examined under microscope. The movements of the microorganisms were taken as a positive result.

Biochemical tests

Indole test

Indole is a nitrogen – containing compound formed from the degradation of the amino acid tryptophan by certain bacteria. Tryptophan present in the culture media was acted by the enzyme tryptophanase and converted into indole, skatol and indole acetic acid. Indole reacted with aldehydes to produce a red coloured product. The microorganisms were grown in tryptophan rich medium and tested for the presence of indole. Tryptophan broth was prepared and a loopful of the culture was inoculated into indole tube and incubated at 37°C for 24 h. Positive results were identified by adding 1 ml of Kovacs reagent and a red coloured ring formed due to the production of indole.

Methyl red test

Methyl red test was used to identify the change in the pH of the medium. Certain microbes fermented glucose to pyruvate and produce mixed acids and other end products. Because of the abundant acid production the final pH of the broth dropped below 4 to 5 which can be detected by pH indicators. MR-VP medium was prepared and 5ml of medium was transferred into the test tubes and sterilized. One loop full of the culture was inoculated into MR-VP medium and incubated at 37°C for 24 h. Formation of red colour by adding one drop of methyl red reagent indicated the positive reaction.

Voges proskauer test

Some organism's fermented glucose; pyruvate and produce butylene glycol and acetone

which were more neutral in nature were noticed. One loop full of the culture was inoculated into MR – VP medium and incubated at 37°C for 24 h. Few drops of VP reagent were added in the 24 h incubated broth. The appearance of bright brown colour indicated positive reaction.

Citrate test

Some of the pathogenic bacteria were capable of utilizing citrate as the sole source of carbon and mono ammonium phosphate as the sole source of nitrogen. Simmon Citrate medium was prepared and sterilized. Then 5ml of medium was transferred into sterile test tube and kept in slanting position. A loop full of culture was streaked on the slant and incubated at 37°C for 24 h. Change of colour from green to blue indicated positive reaction.

Triple sugar iron test

Growth of the organism on the TST slant indicated the type of sugar fermented and in addition, identifying the hydrogen sulphide producer. With acid production the colour of the phenol red indicator turned yellow. An alkaline reaction of the medium was indicated by the purple colour. Production of hydrogen sulphide was indicated by the formation of black colour as hydrogen sulphide combines with ferrous ammonium sulphate. TSI medium was prepared and dispensed 5ml portion into the test tubes and sterilized and allowed it to solidify in the slant position. A loop full of culture was streaked onto the slant and incubated at 37°C for 48 h. The appearance of orange colour to red or yellow and formation of hydrogen sulphide gas indicated the positive result.

Catalase test

Most aerobic organisms were capable of splitting hydrogen peroxide to release free oxygen. The release of oxygen gas can be seen readily by the white bubble if a few drops of hydrogen peroxide were added to the culture. A pinch of organisms or a colony was taken in a sterile stick and dipped in the hydrogen peroxide. Effervescence of hydrogen peroxide allowed that the organism was catalase positive.

Oxidase test

A loop full of culture was rubbed with disc having the reagent tetra methyl paraphenylene

diamine. The disc colour change within 10 seconds was noted. The appearance of blue colour indicated the presence of oxidase enzyme. Peptone water was prepared aseptically and the culture was inoculated and incubated at 37°C for 24-48 h. 2ml of 24 h old culture was removed and introduced into another test tube of 10 ml capacity aseptically. 0.5ml of the Kovacs reagent was added along the side of the test tube to form a layer at the tip. Positive reaction was indicated by the formation of a pink ring at the junction of the solution.

Urease test

A loop full of the isolated colonies was inoculated on urea agar and incubated at 37°C for 18-24 h. After incubation the appearance of pink colouration was taken as positive and recorded.

Coagulase test (Slide test)

A drop of saline (0.85% NaCl) solution was placed on a clean microscopic slide. The test culture was emulsified with it. After emulsification, 1 drop of plasma was placed on the surface of the glass slide. The strain was agglutinated and developed a smooth, milky suspension on the slide.

Antibiotic sensitivity test

A total of 7 bacterial genera were isolated

from hospitalized patients. In vitro sensitivity of all isolates was determined by Kirby Bauer disc diffusion method. A swab was made with the 24 h broth culture uniformly over the surface of the Muller Hinton Agar plate. The antibiotic disc from the container was removed using the sterile forceps and placed it gently on the surface of the inoculated plate. The disc was tapped gently with the forceps to fix in a position. Likewise other antibiotic discs were also placed in the same way on the surface of the culture inoculated agar plates. The Petri plates were incubated at 37°C for 24 h. The antimicrobial activities of different antibiotics were recorded by measuring the inhibition zone.

RESULTS AND DISCUSSION

A total 50 throat samples were screened from patients of various hospitals at Coimbatore. The total microbial strains isolated from patients at various hospitals were illustrated in (Table 1). Of these 40 samples showed positive for the incidence of nosocomial pathogens. The collected samples were streaked on Blood agar, MacConkey agar and EMB agar for the isolation of nosocomial

Table 1. Incident of nosocomial pathogen from the nosocomial infection among patient

S. No	Microorganisms	Number of positive isolates	Percentage of nosocomial isolates
1	<i>Staphylococcus sp</i>	14	35 %
2	<i>Streptococcus sp</i>	2	5 %
3	<i>E.coli</i>	5	12.5 %
4	<i>Pseudomonas sp</i>	3	7.5 %
5	<i>Klebsiella sp</i>	12	30 %
6	<i>Enterobacter sp</i>	2	5 %
7	<i>Bacillus sp</i>	2	5 %

Table 2. Morphological characteristics of various bacterial genera isolated from nosocomial infection.

S.No	Microorganisms	Gram staining	Motility
1	<i>Staphylococcus sp</i>	+ve cocci	Non motile
2	<i>Streptococcus sp</i>	+ve cocci	Non motile
3	<i>E.coli</i>	-ve rod	Motile
4	<i>Pseudomonas sp</i>	-ve rod	Motile
5	<i>Klebsiella sp</i>	-ve rod	Non motile
6	<i>Enterobacter sp</i>	-ve rod	Non motile
7	<i>Bacillus sp</i>	-ve rod	Motile

Table 3. Showing Biochemical characteristics of various nosocomial bacterial pathogens isolated from the patient of various hospital sources

Isolates	Indole	Methyl red	Vogus proskauer	Citrate	TSI	Catalase	Oxidase	Urease	Coagulase	Type of haemolysis
<i>Staphylococcus sp</i>	- ive	+ive	+ive	- ive	A/A	+ ive	+ ive	-ive	+ ive	- ive
<i>Streptococcus sp</i>	- ive	+ ive	- ive	+ ive	-	+ive	-ive	-ive	- ive	- ive
<i>E.coli</i>	+ ive	+ ive	- ive	- ive	A/A	+ ive	- ive	-ive	+ ive	B
<i>Pseudomonas sp</i>	- ive	- ive	- ive	- ive	K/K	+ ive	+ ive	-ive	- ive	- ive
<i>Klebsiella sp</i>	- ive	- ive	+ ive	- ive	K/K	+ive	- ive	+ive	- ive	- ive
<i>Enterobacter sp</i>	- ive	- ive	+ ive	+ ive	A/K	- ive	- ive	-ive	- ive	- ive
<i>Bacillus sp</i>	- ive	- ive	+ ive	- ive	A/K	+ ive	-	+ive	- ive	- ive

Table 4. Showing antibiotic sensitivity of *Staphylococcus sp* against antibiotics

S. No	Antibiotics	Symbol	Strength	Diameter of zone of inhibition		Appearance of zone	Remark
				Sensitive	Intermediate	Resistant	
1	Ampicilin	Am	10	8	10-17	18	Moderate Resistant
2	Amoxycilin	Ac	10	8	10-17	18	Susceptible
3	Cefazolin	Cz	10	8	10-17	18	Susceptible
4	Gentamicin	G	10	8	10-17	18	Highly Resistant
5	Neomycin	N	10	8	10-17	18	Highly Resistant
6	Rifampicin	R	10	8	10-17	18	Sensitive
7	Tetracycline	T	10	8	10-17	18	Susceptible
8	Vancomycin	Va	10	8	10-17	18	Sensitive

pathogens. The bacterial genera of nosocomial pathogens primarily comprised of *Staphylococcus sp*, *streptococcus sp*, *E.coli*, *Bacillus sp*, *Pseudomonas sp*, *Klebsiella sp* and *Enterobacter sp* were isolated and identified from throat samples collected from patients. Among the various bacterial isolated *Staphylococcus sp* and *Klebsiella sp* found to be predominant microflora and this was followed by *Streptococcus sp*, followed by *E.coli*, followed by *Pseudomonas sp*, *Enterobacter sp*, and *Bacillus sp*. The similar trend was observed by several authors.

The morphological characteristics of various bacteria isolated from nosocomial pathogens was identified by Gram staining and motility test are presented in (Table 2). The present study showed the dominance of non –motile gram positive cocci belongs to the genera of *Staphylococcus sp* and *streptococcus sp*. The present study also revealed the occurrence of *E.coli* and *Pseudomonas sp* which were motile gram negative rod. Non motile gram negative rod belongs to the genera of *Klebsiella sp* and *Enterobacter sp*. Motile gram positive rod belongs to the genera of *Bacillus sp*.

Antibiotic sensitivity test was employed to test the susceptibility of *Staphylococcus* against various antibiotics and their result shown in table 4 and fig 1. The result of the present study showed that the dominant nosocomial pathogens, *Staphylococcus sp* isolated from patients were found to be highly resistant to Neomycin and Gentamicin. Moderate resistant to Ampicilin, Rifampicin, and Vancomycin. Table 5 and fig 2 illustrates that the antibiotic sensitivity of *Klebsiella sp* isolated from various hospital sources against different antibiotics. It had been observed that these strains were highly resistant to Neomycin. Moderate resistant to Ampicilin, Amoxycilin, Cefazolin and Gentamicin.

Ram *et al.*, (2000) isolated *Staphylococcus aureus* and *E.coli* from blood and urine samples collected from blood and urine samples collected from hospital attached medical college at Ludhiana. Richard *et al.*, (2000) found that *Staphylococcus aureus* was the most common pathogen present in medical surgical intensive care unit in united states Szewczyk *et al.*, (2000) reported that the presence of predominant *Staphylococcus sp* in intensive care unit . This coagulase negative

Table 5. Showing antibiotic sensitivity of *Klebsiella sp* against antibiotics.

S. No	Antibiotics	Symbol	Strength	Diameter of zone of inhibition		Appearance of zone	Remark
				Sensitive	Intermediate	Resistant	
1	Ampicilin	Am	10	8	10-17	18	Moderate Resistant
2	Amoxycilin	Ac	10	8	10-17	18	Sensitive
3	Cefazolin	Cz	10	8	10-17	18	Highly Resistant
4	Gentamicin	G	10	8	10-17	18	Moderate Resistant
5	Neomycin	N	10	8	10-17	18	Highly Resistant
6	Rifampicin	R	10	8	10-17	18	Susceptible
7	Tetracycline	T	10	8	10-17	18	Susceptible
8	Vancomycin	Va	10	8	10-17	18	Sensitive



Fig. 1. Activities of Antibiotics against Drug-Resistant *Staphylococcus sp*



Fig. 2. Activities of Antibiotics against Drug-Resistant *Klebsiella sp*

Staphylococcus sp causes a significant number of infections especially in immunocompromised patients including premature neonates.

In the present investigation *Streptococcus* was found to be predominant organisms isolated from the throat swab of patients. Dimibile *et al.*, (1990) observed that the $\hat{\alpha}$ - streptococci was isolated from the infected patient *Streptococcus sp* was not a well recognized cause of soft tissue infection. In our present study, *Klebsiella sp* was the fifth predominant organism from the throat swabs of patients. As observed in the present investigation. Paniara *et al.*, (2002) isolated *Klebsiella sp* strains

from ICU. Durmaz *et al.*, 2000 observed that Nosocomial Infections are common in patients.

Staphylococcus sp was resistant to Amoxycillin, Rifampicin, Cefazolin, Tetracycline and Vancomycin. The similar trend was observed by several workers. According to Szewczy *et al.*, (2000) found that *Staphylococcus aureus* were resistant to several antibiotics and 97% were resistant to Methicillin. Some strains were susceptible to Pencillin and Amoxycillin. In contrast *Streptococcus epidermidis*, a notable Nosocomial pathogen in some Intensive Care Unit, had developed very diverse pattern of antibiotic resistance. The genuses determine antibiotic

resistance is often carried by extrachromosomal plasmids that can be transferred among bacterial species. In the present study the genus *Klebsiella* *sp* is found to be resistant to Ampicilin and Cefazolin .Paniara (2000) isolated 7 multiresistant *Klebsiella* *sp* species from ICU and these isolates had different levels of resistance to β lactum. In the present study the genus *Enterobacter* was found to be resistant.

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