Research Article

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ANTIBIOTIC SUSCEPTIBILITY OF INFECTIOUS NOSOCOMIAL MICROORGANISMS

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Abstract: Infectious diseases are the leading cause of death across the world. Evolution of antimicrobial drug resistant bacteria is a major health concern and extremely difficult to overcome. Nosocomial infections encompass all the clinical infections that do not originate from patients, originally admitting diagnosis. Critically ill patients have greater risk of developing nosocomial infections with resistant strains. Its incidence in developing nations is 5-10%. However, in India one in four patients admitted in hospital acquire nosocomial infections. These incidences are 4-5 times greater in general ward. In the present investigation strains of *E. coli, Staphylococcus, Klebsiella, Enterobacter* were isolated and identified based on morphological and biochemical tests, and were tested for their antibiotic susceptibilities. All the isolates were resistant to ampicillin and penicillin; however, they were sensitive to streptomycin, chloramphenicol and tetracycline. This study is an attempt to detect the presence of antibiotic sensitive / resistant pathogens in government hospital.

Keywords: Antibiotic Susceptibility, Govt. Hospital, Nosocomial bacteria.

INTRODUCTION

Nosocomial infections remain a major global concern, the most important and frequent mode of transmission of nosocomial infections is divided into two sub groups, direct contact and indirect contact (Pena *et al.,*). They constitute a major public health problem worldwide; increasing antibiotic resistance of pathogens associated with nosocomial infections has also become a major therapeutic challenge for physicians (Vogt and Dippold).

Hospital acquired infections occur in about 5% of all patients admitted to hospital. Nosocomial infections result from interaction of several factors such as microorganisms in the hospital environment, the compromised immune status of the host and the chain of transmission in the hospital. *Staphylococcus* has been reported as the most important and wide spread hospital pathogen. This has been implicated as the most common cause of surgical wound infections and pneumonia and the second most common cause is blood infection, other pathogens common in the hospital environment include *E. coli, Enterocoocus, Pseudomonas, Candida, Klebsiella* (Shilpa jalalpour and Abdul Ghaffar ebadi).

Bacterial infection is a common cause of hospital acquired infections; most of the bacterial infections can be easily diagnosed and treated with few exceptions. The emergence and spread of resistant bacteria is complicating the treatment of serious hospital acquired infections and threatening to create species resistance to all available agents (Bartosova *et al.,*).

Antibiotic resistance among bacteria is becoming a serious problem throughout the world. It is said that the evolution of bacteria towards resistance is unavoidable because it represents a particular aspect of the general evolution of bacteria that is unstoppable. Thus, in the present study, an attempt has been made to know the current status of antibiotic sensitive/ resistant pattern of common bacterial isolates in the general ward of government hospital, Hyderabad.

MATERIALS AND METHODS

Isolation and characterization of Nosocomial microorganisms

Medically important selective media was prepared and sterilized in an autoclave. The petriplates were exposed to hospital environment in the general ward by petriplate exposure method at different time intervals starting from 5,10,15,20,25 and 30 minutes respectively. Then all the plates were incubated at 37° C for 24 to 48 hours and counted to know the total viable cells. Bacterial colonies were purified by taking single colony each time in a streak plate method on medically important selective media repeatedly; at least 7 times

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until plate contain single type of colonies. These purified colonies were further subjected to morphological and biochemical characterization (Ariffin *et al*., Durmaz *et al*., Baron *et al*., Barrow and Felthan and Bartosova *et al.,*).

Antibiotic susceptibility test

Antibiotic susceptibility test was performed by Kirby Bauer's disc diffusion method on Muller Hinton agar medium (high media, Mumbai). In accordance with standards of clinical laboratory standards institute (CLSI) formally national committee clinical laboratory standards (NCCLS) guidelines. The concentration of various antibiotics for tested organism viz. penicillin (15 & 30 µg), ampicillin (15 & 30µg), chloramphenicol (15 & 30 µg), tetracycline (15 & 30µg), streptomycin (15 & 30µg) respectively. The antibiotic diffuses from a paper disc or a small cylinder into an agar medium that contains test organism. A common application of the method of Kirby Bauer disc test is that the paper disc contains known concentration of antibiotics. Antibiotics are applied to the surface of the Muller-Hinton agar plates and were incubated at 37° C for 24-48 hours, after overnight incubation, the susceptible of resistance isolates were identified in the presence of zone of inhibition around the antibiotic discs. The inhibition zones were measured in mm.

Statistical analysis

By using Kirby-Bauer Disc Diffusion Method the antibiotic susceptibility test was performed for the four different isolates. The quantitative data was checked for completeness and coded (Kirkl and Briggs and http://www.who.int/csrresources/ publications/whocdscreph 2002.pdf.accessed)

The antibiotic sensitivity values in triplicate are subjected to statistical analysis by using SPSS (statistical software package) followed by analysis of mean, standard deviation and probability test ($P < 0.05$).

RESULTS

Nosocomial infections are those infections acquired as a result of treatment in a hospital or health care service providing center. These infections usually appear 48 hours or more after hospital admission or within 30 days after discharge. Nosocomial infections have been a major problem to health care delivery. These often result in prolonged recovery of patients and even death when not treated early (Oyetayo and Miiori http://www.who.int/csrresources/ publications/whocdscreph2002.pdf.accessed) Different types of bacteria, fungi and viruses have been implicated in the development of nosocomial infections (Jarvis and Martone). Several species of microorganisms have been isolated from different hospitals across the world. Even though some of these organisms were not known for causing recalcitrant nosocomial infections, they are opportunistic pathogens and hence pose a challenge to patients especially those with immune-compromised conditions.

For sample collection the medically important selective media were prepared and the plates were exposed to the general ward in the ESI Hospital, Hyderabad, Andhra Pradesh. The plates were incubated at 37°C for 24-48hrs. The bacterial isolates were purified by streak plate method to obtain pure colonies. The isolates were identified based on morphological and biochemical characteristics and the results of the selected bacterial isolates are summarized in table 1 and 2. The isolates were identified as Staphylococcus, *E. coli*, klebsiella and Enterobacter.

By Kirby-Bauer Disc Diffusion Method the antibiotic susceptibility testing was performed for the four isolates. Antibiotic susceptible results depict that ampicillin and penicillin are resistant to our isolates where as tetracycline, streptomycin, chloramphinicol is sensitive to our bacterial isolates. The quantitative data was checked for completeness and coded (Kirkl and KB *et al*., Pena *et al.,* Shilpa jalalpour and Abdul Ghaffar ebadi. V.O Oyetayo and R. Miiori., Vogne C *et al.,* Vogt, R.L., and Dippold, L http://www.who.int/csrresources/ publications/whocdscreph 2002.pdf.accessed). The different types of antibiotic susceptible triplicates values are subjected to statistical analysis by using SPSS (statistical software package) followed by mean, standard deviation and probability test ($P < 0.05$).

Table 1: Morphological characterization of Nosocomial isolates

S.No	Characterization	Isolate 1	Isolate 2	Isolate 3	Isolate 4
1.	Colony morphology	Yellow, slimy circular	Round, small enteric	Round, smooth, enteric	Shiny colony
2.	Shape of the colony	Cocci	Rod	Rod	Rod
3.	Gram's stain	Positive	Negative	Negative	Negative
4.	KOH Test	Negative	Positive	Positive	Positive
5.	Motility Test	Negative	Positive	Negative	Positive

Table 2: Biochemical characterization of Nosocomial isolates

S.No.	Biochemical Test	Isolate 1	Isolate 2	Isolate 3	Isolate 4
1.	Indole	Negative	Positive	Negative	Negative
2.	Methyl Red	Positive	Positive	Negative	Negative
3.	Voges Proskauer	Negative	Negative	Positive	Positive
4.	Simmon's citrate	Negative	Negative	Positive	Positive
5.	Carbohydrate fermentation:				
5.a.	Glucose - Acid & Gas	Positive	Positive	Positive	Positive
5.b.	Sucrose - Acid & Gas	Positive	Positive	Positive	Positive
5.c.	Lactose - Acid & Gas	Positive	Positive	Positive	Positive

Table 3: Antibiotic susceptibility pattern at 15µg concentration of various antibiotics

Media	Organism	Penicillin	Ampi-cillin	Chloram-pheinicol	Tetra-cycline	Strepto-mycin
MSA	*Staphylo-coccus*	0.6±0.01	0.9±0.05	1.6±0.01	1.68±0.01	4.0±0.01
MAC	*E. coli*	0.71±0.05	0.6±0.02	1.3±0.01	0.96±0.01	3.3±0.01
EMB	*Entero-bacter*	0.8±0.01	0.9±0.01	1.73±0.05	1.83±0.01	3.03±0.01
DCA	*Klebsiella*	0.8±0.01	0.8±0.03	1.7±0.01	1.65±0.01	4.0±0.01

MSA – Mannitol salt agar; MAC – Mc Conkey's agar; EMB – Eosine methylene blue agar; DCA – Deoxy cholate agar

Table 4: Antibiotic susceptibility pattern at 30 µg concentration of various antibiotics

Media	Organism	Penicillin	Ampi-cillin	Chloramph-inicol	Tetra-cycline	Strepto-mycin
MSA	*Staphylo-coccus*	1.2±0.01	1.3±0.01	3.3±0.01	2.93±0.01	6.93±0.03
MAC	*E. coli*	1.3±0.02	1.4±0.04	2.7±0.01	2.06±0.01	6.33±0.03
EMB	*Entero-bacter*	1.65±0.02	1.5±0.01	3.53±0.05	4.06±0.02	6.03±0.03
DCA	*Klebsiella*	1.65±0.05	1.8±0.05	3.5±0.01	3.4±0.01	6.8±0.01

MSA – Mannitol salt agar; MAC – Mc Conkey's agar; EMB – Eosine methylene blue agar; DCA – Deoxy cholate agar

DISCUSSION

Nosocomial infections occur worldwide and affect both developed and developing countries (World health organization. Ed., A Practical Guide) Many of these infections are associated with microorganisms that are resistant to antibiotics and can easily spread by hospital personnel (B. Durmaz) Guidelines for antibiotic therapy can be helpful for clinicians to select more appropriate antibiotics for effective treatment and prevent the development of drug resistance. Hospital associated infections have been linked with many factors among which the critical microbial quality of the air of general ward of government hospital. This infection occurs in 5% of all acute care hospitalization in India and has been reported to be responsible for the death of one out of every five thousand patients attending a hospital (Ariffin *et al*., B. Durmaz *et al*., Baron *et al*., Barrow et al., Bartosova *et al.,*).

The microbiological quality of air in hospitals is as much of an issue as in other type of buildings, with increased emphasis because of the potential severity of the consequences of nosocomial infections. Many patients are actually at increased risk of infection while in the hospital. The problems of nosocomial infection are generally largest in older hospitals which may have large wards and poor or no mechanical ventilation and the situation is even more difficult in developing countries (Archibald *et al.,).*

The results from this study showed that the government hospital had a higher degree of contamination with airborne micro flora and in indoor air rather than the private hospital. These high rates in the government hospital might be attributed to the age, poor hygienic condition, and low degree of cleanliness and minimal application of disinfection procedures against airborne bio-contaminants. The high number of visitors that commonly enter the patients room and the amount of materials brought from outside by the visitors, such as food, fruits and flowers were more common in the patients rooms; these are the recognized source of hospital contamination. Intensive care units are high risk areas for infections caused by antibiotic resistant bacteria that may spread to other clinical areas of the hospital (Courvalin P *et al*., Danishta *et al*., Jarvis WR and Martone WJ., Kanouff *et al*., Kirkl *et al.,*).

The number of microorganisms in the operation theaters and neonatal ward was extremely low. This was anticipated due to the high sanitary standards in that areas compared to the other hospital areas.

CONCLUSION

It is concluded that in Govt. Hospital there is an involvement of both gram positive and gram negative bacteria for cause of nosocomial infections. Nosocomial microbes are resistant to commonly used antibiotics like penicillin and ampicillin, but sensitive to streptomycin, tetracycline and chloramphenicol etc. The lack of knowledge regarding hospital microbiota and the improper monitoring of antimicrobial therapeutic can lead to microbial resistance and favoring selective pressure to developing resistant strains.

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