ISOLATION AND CHARACTERIZATION OF VARIOUS PATHOGENS PREVALENT IN THE ENVIRONMENT OF HOSPITAL

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ABSTRACT

This article presents review on the attention of hospital acquired infections that is necessary to be cured in order to achieve public health goals with optimal efficiency and sustainability. Extensive study was carried out to determine prevalence of bacteria in nosocomial infections at Govt. Hospital, Dehra Dun. During study air samples were collected for hospital toilets and corridors. Seven isolates were obtained. After characterization, isolates were identified to be Staphylococcus. Sanitation, Mitigation and Disinfection are the strategies to control this morbidity but still rapid infection occurs therefore safe procedures should be carried out by infection control organization.

KEYWORDS: Characterization, Hospital Environment, Pathogens, Review

INTRODUCTION

Nosocomial infections are also known as hospital acquired infections which are one of the main causes of mortality and morbidity in hospitalized patients at present time, leading directly or indirectly to an enormous increase in the cost of hospital care and to the emergence of new health hazards for the community. Hospital acquired infections are caused by bacteria (e.g. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Clostridium* etc), Virus (e.g. H.I.V., Hepatitis B, C, D), Parasites and fungi. Most of the Nosocomial infections are inevitable risk related to treatment (Abdel Fattah., 2003). Prevalence survey on hospital acquired infections can give us general pictures on the size of problem and it is documented that repeated surveys are told for monitoring the trend of hospital acquired infections within the individual hospital (Burgner et al., 1996).

SPREAD

Hospital acquired infections can be caused by bacteria, viruses, fungi and parasites. Microorganisms may find many reservoirs where they reside multiply and spread from one place to another. These microorganisms may already be present in the patient’s body or may come from the environment, contaminated hospital equipment, health care workers or other patients. Even prior to the latest studies there was little doubt that transmission of microorganisms from the health care workers is still the main cause of nosocomial infection (Pittet et al., 1999).

MICROORGANISMS RESPONSIBLE FOR NOSOCOMIAL INFECTION

Bacteria

Large numbers of bacteria are responsible for hospital acquired infections. Nosocomial bacteraemia in intensive care units is due to *Pseudomonas aeruginosa*. *Staphylococcus* is a genus found almost everywhere including in the soil and on the skin of many animal species. *Staphylococcus aureus* is commonly found on skin and in nasal passages of humans. *Staphylococcus aureus* infections often fatal in the pre-antibiotic era, now typically respond to a variety of antimicrobial agents (Skinner., 1941). However the spread of multidrug resistant strains of *Staphylococcus aureus* in health care settings,
particularly MRSA (*Methicillin resistant Staphylococcus aureus*) have made these infections more difficult to treat (Cosgrove et al., 2003). Certain *Methicillin resistant Staphylococcus aureus* strains appear to be spreading in the community settings (Herold et al., 1998). In hospitals bacteria, viruses or fungi can cause pneumonia but most hospital acquired pneumonia is caused by *Staphylococcus aureus* and gram negative opportunists (Inglis et al., 1993). Research from the veterinary community on *Methicillin resistant Staphylococcus aureus* infection and colonization of animals and pets has identified yet another reservoir of *Methicillin resistant Staphylococcus aureus* that is transmissible to humans (Wesse et al., 2006).

**Virus**

Patients and hospital personnel may acquire infection by HIV and hepatitis B, C, D viruses through contact with blood positive for these viruses from patients and blood donors (Parker, 1978).

<table>
<thead>
<tr>
<th>Table 1: Types of Nosocomial Infections</th>
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<tr>
<td>Surgical Site Infection</td>
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<td>Any purulent discharge abscess, or spreading cellulites at the surgical site during the month after the operation</td>
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<tr>
<td>Urinary Infection</td>
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<tr>
<td>Positive urine with at least 10 bacteria / ml with or without clinical infection</td>
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<tr>
<td>Respiratory Infection</td>
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| Respiratory symptoms with at least two of the following signs appearing during hospitalization - 
  - cough 
  - purulent sputum 
  - new infiltrate on chest radiograph consistent with infection |
| Vascular Catheter Infection            |
| Inflammation, lymphangitis or purulent discharge at the insertion site of the catheter |
| Septicemia                             |
| Fever or rigors and at least one positive blood culture |

Surgical site infections are also frequent; the incidence varies from 0.5 to 15 % depending on the type of operation and underlying status.

**ANTIBIOTIC RESISTANCE**

Today the growing problem of antibiotic resistance in bacteria is of primary concern. This is because bacteria resistant to a number of antibiotics commonly prescribed by heath care personnel. Penicillin resistant Staphylococcus were common until penicillin had been freely available in hospitals for several years (Barber., 1947). Staphylococci develop resistance to erythromycin more readily than to any other antibiotic except streptomycin (Hobson et al., 1956).

*Methicillin resistant Staphylococcus aureus* has become a nosocomial problem in hospitals for both children and adults (Karamat et al., 1996). Overuse of antibiotics in modern health care has lead to certain ESBLs (extended spectrum beta lactamase) enzymes that destroy beta lactam antibacterials. As with *Methicillin resistant Staphylococcus aureus*, isolation and appropriate barrier precautions will be required for patients who are colonized or infected with ESBL producing bacteria. Many of the strains of Staphylococcus show not only resistance but also multidrug resistance. The common use of avoparcin as a growth promoter, in animal husbandry and its possible role in the development of vancomycin resistant Enterococci in humans (Denis and Spelman., 2002).

**MATERIALS AND METHODS**

Extensive study was carried out to determine the prevalence of pathogens in nosocomial infections or Hospital
acquired infections at Govt. Hospital, Dehra Dun in India. Air Samples of hospital environment were evaluated for pathogens. Methods of this research follow different steps.

- Sample collection
- Pure culturing
- Maintenance of bacterial isolates
- Characterization of bacterial colonies
  - Gram staining
  - Study on selective media
  - Biochemical identification
  - Detection of virulence factors
  - Antibiotic sensitivity test
- Preventive measures

Air Samples of hospital environment were evaluated for pathogens with Gram staining, biochemical testing, tests for detection of virulence factors and antibiotic sensitivity tests. Samples of Hospital environment were obtained from hospital general ward and toilet by exposing plates of Dettol agar, Mac Conkey agar and Blood agar. Four plates were exposed in general ward and four in toilet for ten minutes and brought to the laboratory without any delay. Samples were incubated at 37°C for 24 hours. On next day growth was observed only on Blood agar plates. Isolates were named as C1, C2, C3, C4, T1, T2 and T3. The isolates were routinely maintained on nutrient agar slants, stored at 4°C. Then Gram staining of all colonies from seven plates of Blood agar was done. The cultures were streaked on Mannitol salt agar (MSA) and incubated at 37°C for 24 hours. Biochemical identification was done with IMVic test, TSIA test, catalase test, oxidase test and nitrate test. Detection of virulence factors was done with protease test, esterase test, phospholipase test, coagulase test and DNAse test. Then antibiotic step was checked against Vancomycin, Methicillin, Erythromycin, Amoxycillin and Novobiosin by disk diffusion method.

RESULTS

During study air samples were collected by plate exposure method for hospital toilets and corridors and then cultured for isolation of bacteria. Seven isolates were obtained. All isolates were found to be gram positive, coccus in bunches. Further isolates were characterized by biochemical tests. All isolates were found to be positive for MR, VP, catalase and nitrate. On streaking on MSA four isolates gave fermentative colonies. When compared with Bergey’s manual the isolates were identified to be Staphylococcus. Virulence properties of isolates were also determined. All isolates were found to be positive for esterase, phospholipase, coagulase and DNAse. After identification in vitro antibiotic sensitivity was checked for recovered isolates. Antibiotics belonging to different classes were used. Five isolates of Staphylococcus were resistant to vancomycin and all were resistant to methicillin. Some isolates showed resistance to these antibiotics.

DISCUSSIONS

Hospital Acquired Infection (HAI) or nosocomial infection occurs when the pathogen interacts with the susceptible host during his/her stay at hospital. Occurrence of disease depends upon the competition between the virulence
factors and host defense mechanisms. When the pathogen succeeds in evading the host immune response, then only it is able to cause the disease. In the present study air samples were collected and the pathogens isolated were identified biochemically. 90 % results of the isolates matched with the bacteria *Staphylococcus*. Members of the genus *Staphylococcus* are differentiated by the ability to clot plasma by the action of the enzyme coagulase. Coagulase exists in two forms Bound coagulase which is bound to the cell wall and free coagulase which is liberated by the cell wall. Bound coagulase absorbs fibrinogen from the plasma and alters it so it precipitates on the staphylococci, causing them to clump resulting in cell agglutination. Free coagulase reacts with a substance in plasma to form a fibrin clot.

*Staphylococcus* gave negative results as it does not possess any extracellular enzyme as invasions. Another soluble protein involved in invasion is phospholipase and lecithinase which are being produced by *Staphylococcus*. It acts synergistically to break down the lipids. Hemolysins contribute to invasion through their cytotoxic effects on neutrophils, lymphocytes and other eukaryotic cells. Greenish blue color of copper soap of fatty acid on egg agar confirms lipolysis and hence the presence of phospholipase is confirmed.

As antibiotic sensitivity is concerned *Staphylococcus* is notorious for its resistance to antibiotics and is therefore a particularly dangerous and dreaded pathogen. Hospital strains of *Staphylococcus aureus* are usually resistant to a variety of different antibiotics. The terms MRSA refers to *Methicillin resistant staphylococcus aureus*. Methicillin resistance is widespread and most methicillin resistant strains are also multiple drugs resistant. A plasmid associated with vancomycin resistance has been detected in *Enterococcus faecalis* which can be transferred to *Staphylococcus aureus* in the laboratory and it is speculated that this transfer may occur naturally (e.g. in the gastrointestinal tract). In addition *Staphylococcus aureus* exhibits resistance to antiseptics and disinfectants, such as quaternary ammonium compounds, which may aid its survival in the hospital environment. The lab isolate was also found to be methicillin resistant. It showed partial inhibition with erythromycin.

**CONCLUSIONS**

The results showed high incidence of vancomycin and methicillin resistant Staphylococcus species in hospital air. Hospitals and medical institutions should know the prevalence of bacteria and pattern of their susceptibility to antibiotics in their institution from time to time so that nosocomial infections can be prevented by administration of proper antibiotics to the patients before the surgery or any other clinical treatment.

**REFERENCES**

6. Denis W Spelman, 2:Hospital-acquired infections MJA 2002:176(6); 286-291


