Antibiotic Resistance Bacteria in Tertiary Hospitals in Chittagong, Bangladesh

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Abstract— *Nosocomial infections (HAI) are major cause for mortality and morbidity worldwide. In low income countries, data suggests 6.5% - 33% of patients have HAI with pneumonia being the most frequent..Antibiotic resistance is highly prevalent in developing countries due to self medication, easy availability and poor regulatory controls. Clinicians have been left with limited antibiotic drug options for the treatment of bacterial infections due to escalated rates of resistance. This comparative study aimed to identify microorganisms from hospital surfaces in two major tertiary care hospitals in Chittagong, Bangladesh. It also identifies antibiotic susceptibility of the samples to antibiotics commonly used in Bangladesh. Samples were collected by swabbing different environmental surface around patients in both hospitals. Identification of bacteria was done by culturing in nutrient media and various common biochemical techniques. Antibiotic sensitivity was determined by disk diffusion method. During the study, 27 samples were collected from different surfaces in different wards of the hospitals. The predominating organisms were Streptococcus, Staphylococcus, Bacillus, Pseudomonas and Serratia. The isolates of organisms showed high level of resistance to commonly used antibiotics especially a fourth generation cephalosporin, cefepime. In addition, antibiotic sensitivity tests showed small colonies or film of growth within zone of inhibition of some of the samples known as "satellite colonies". The study identified bacterial isolates responsible for HAI in tertiary hospitals and their susceptibility to antibiotics. Further research is currently being conducted on understanding the satellite colonies some of the isolates from hospital surface swabs have exhibited.*

Keywords— *Antibiotic Resistance; Bangladesh; Bacterial Isolate; Hospital Acquired Infection; Staphylococcus, Tertiary Hospital.*

I.   INTRODUCTION

One of the most common causes of mortality and morbidity is due to bacterial infections acquired from hospitals while getting treatment also known as Hospital Acquired Infections (HAI) [1]. HAI is a major but preventable infection to patients hence there are HAI surveillance systems in developed countries such as National Healthcare Safety Network (NHSN) in USA and Healthcare Associated Infections Surveillance Network (HAI-Net) in Europe[2]. In 2011, 722,000 patients were reported to have HAI in USA. There is insufficient data representing HAI in low income countries like Bangladesh[3]. The infections acquired from hospitals represent major health concern worldwide. Significant role in spreading of evolving infections are played by the hospitals [4]. A study by Bhuiyan *et al.* 2012 has shown that hospital patients in Bangladesh are highly susceptible to endemic infections [5].Contamination of surfaces of the environment acts as the potential reservoir for pathogens to persevere and cause infection to vulnerable patients[6]. Environment of a hospital is a reservoir for a wide variety of microorganisms and the surfaces near patients such as bed, tray, floor, washroom, incubators etc are often heavily contaminated and are responsible for the spread of pathogenic microbes. These contaminated surfaces facilitate the transmission of pathogens like Gram positive *Staphlylococcus aureus* (MRSA) methicillin resistant, *Enterococcus*, Gram negative pathogens like *Pseudomonas, Klebsiella, and Acinetobacter* etc. In a study by Schmidt et al., it was shown that microbes can persist for weeks and have the ability to colonize any surface and on steel surfaces in hospitals. Length of nosocomial pathogen persistence on a surface prolongs its potential to be a source of transmission to susceptible patients[6].

Several studies in Bangladesh have also shown that the surfaces contaminated with pathogens are a common source of transmission of the infections[7]. A study conducted on tertiary hospitals in Dhaka, Bangladesh reported the following bacteria, *E. coli, Pseudomonas species (spp.), Proteus spp.*, *S. aureus, Klebsiella and Acinetobacter spp* responsible for HAI i.e. postoperative wound infection, urinary tract infections (UTI) and diabetic wounds[8]. In another study, bacterial isolation from door handles of a Dhaka hospital washroom swabs reported similar bacteria including *Bacillus spp.*, Fecal coliform and *Micrococcus spp.*[9]. Afroz et al. confirmed similar results in a different tertiary care hospital in Dhaka[10]. This shows that the environment in healthcare facilities present microbes' common to such facilities.

Furthermore, nosocomial infections are a threat to health care as antibiotic sensitivity of the microorganism causing infections has become resistant to first generation of cephalosporin and beta lactam antibiotics[8]. Factors which are responsible for the spread of resistance to antibiotics are unregulated administration and manufacture of antimicrobials, ease of access to antimicrobials, improper or not completion of antimicrobial therapy and self-prescribed antimicrobial treatment[11]. Resistance of the microbes to commonly used antibiotics further exaggerates the incidence of HAI.

The study aims to identify the bacteria commonly present in environment of hospitals in Chittagong, Bangladesh and the level of antibiotic susceptibility of the sample bacteria to commonly used antibiotics in Bangladesh.

II. MATERIALS AND METHODS

A. Study Design

The study was conducted in Chittagong Medical Hospital and Chattagram Ma-O- Shishu Hospital. The study was carried out during normal hospital activities.

B. Sample Collection and Bacterial Analysis

Samples were collected by using a sterile cotton swabs. Samples were collected from surfaces of different wards environment surfaces surrounding hospitalized patients. The following surfaces were chosen: floors, bed, balcony, balcony bed, tray, washroom, incubators and cabinets. The collected samples were grown in nutrient media by incubating for 24 hours at 37^0C. The bacteria were then identified by conventional biochemical techniques such as Gram Staining, oxidase or catalase test etc.

C. Antibiotic Susceptibility

After the completion of bacteria identification, antibiotic susceptibility test was carried out. The antibiotics chosen to carry out the test is of broad spectrum and most commonly prescribed antibiotics in Bangladesh. The antibiotic susceptibility test was carried out by applying bacterial inoculums of approximately $1–2\times10^8$CFU/mL to the surface of agar plate. Antibiotic disks were placed on the inoculated agar surface (Figure 2). Plates were incubated for 16–24 h at 35°C prior to determination of results. The zones of growth inhibition around each of the antibiotic disks are measured to the nearest millimeter. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. The zone diameters of each drug were interpreted as susceptible, intermediate or resistant according to the zone of inhibition obtained.

III. RESULTS

A. Bacterial Isolation from Surface Swabs

There were 11 samples from Chittagong Medical Hospital [CMH] and 16 samples from Chattagram Ma-O Shishu Hospital. The samples from CMH were collected from two wards, Surgery and Gynaecology whereas the samples from Ma-O Shishu Hospital were collected from four wards, Surgery, Pathology, Gynaecology and Neonatal including Neonatal ICU. Among the 27 samples collected and gram stained, there were more gram positive than gram negative bacteria. The study found 10 gram positive cocci , 5 gram positive rods. There were also a mix of gram positive bacteria and gram positive/negative bacteria which includes 6 gram positive mix of rods and cocci, 2 gram positive/negative rods, 1 gram positive/negative cocci and 3 gram positive/negative rods and cocci.

The list of bacteria that tested positive based on the biochemical test that were conducted which includes, catalase, oxidase, esculin, fermentation, citrate, MR-VP tests etc. is shown in Table 1. Color change of Mannitol Salt Agar (MSA) to yellow indicating growth of *S. aureas* is shown in Figure 1.

TABLE 1. List of Bacterial Isolates Identified from Samples

Gram Positive	Gram Negative
Streptococcus	Pseudomonas
Pediococcus	Serratia
S. aureas	
Coagulase negative Staphylococcus	
Bacillus	

Figure 1. Bacterial growth in Mannitol Salt agar (MSA) showing Positive results

B. Susceptibility of Bacterial Isolates to Antibiotics

The bacterial isolates were tested for susceptibility to antibiotics commonly used in Bangladesh [12].The four antibiotics used were Vancomycin (VA), Tetracycline (TE), Cefepime (CPM), Ciprofloxacin (CIP). The study based the results on the following interpretation: Resistant (10< mm), Intermediate (11-15mm), Susceptible (16> mm) [13]. **Table 2** presents the data on susceptibility of the bacterial isolates to the antibiotics.

TABLE 2. Susceptibility of Isolates to Antibiotics

Antibiotics/Category	Resistant 10 or less (mm)	Intermediate 11-15 (mm)	Susceptible 16 or more (mm)
CIP	0	3	24
CPM	27	0	0
TE	1	9	17
VA	3	3	21

Figure 2. Bacterial Plates Showing Antibiotic Resistance

Among the 27 samples, 13 samples showed small number of colonies or a film of bacteria within the zone of inhibition as shown in Figure 2.

19

IV. DISCUSSION

Bangladesh, a low income country, is susceptible to infectious disease due to a high density population, lack of hygiene awareness, inefficient regulatory authority on infection control and most importantly overuse of antimicrobial agents[14]. As a result, the ability of microorganisms to transmit via air, fomites, droplets or direct contact with bodily fluids is higher in hospitals with limited resources[4].

From our list of identified bacteria isolates [Table 1], the common infectious bacteria most responsible for HAI include Gram positive *Streptococcus*, *S. aureas*, coagulase negative *Staphylococcus*, Gram negative *Pseudomonas* and *Serratia*. These has been previously isolated from HAI including UTI, pneumonia, postoperative wound infections, intravenous pain control fluids among others[15, 16, 17].

There have been many researches evaluating the correlation between antimicrobial use and development of resistance to bacteria[18]. According to Rahman et al., microbes showed resistance to antibiotics primarily in hospitals which then extended to the community[19]. The antimicrobial sensitivity pattern conducted in this study reflects the emergence of resistance against antibiotics commonly used in Bangladesh.

Among the 27 isolates, all were resistant to cefepime, a fourth generation of Cephalosporin. This may be the result of the widespread use of cephalosporin antibiotic in Bangladesh[9]. There were 3 isolates resistant to vancomycin and 1 isolate resistant to tetracycline. Ciprofloxacin shows the most susceptibility to 24 isolates compared to vancomycin susceptible to 21 isolates. This is also consistent in research by Hafsa et al. and Faiz et al. who found all bacterial isolates resistant to Cefepime but also showed resistance to ciprofloxacin [9, 14]. In addition, there were 13 bacterial isolates that showed small colonies or film of growth within the zone of inhibition. The study ruled out contamination as some plates clearly showed this phenomenon and not others. These colonies known as "satellite colonies" shows there is some resistance to the antibiotics or the bacteria may be of new strain[20]. For this, further research is currently being conducted to isolate these particular satellite colonies, subculture them and re-identify and test them.

Resistance to microbes that cause infections can lead to greater morbidity and mortality. It is important to identify the common causative microorganisms and source of infection for the prevention and control of HAI in hospitals. This study was conducted in major tertiary care hospitals in Chittagong, Bangladesh. Due to limited studies mostly conducted in tertiary hospitals in Dhaka, more emphasis must be placed on research in identifying resistant patterns to strains of microbes particular to health service centers in Bangladesh. The study is limited in such that it has only identified microbes from surface swabs which may lead to some of the common known HAI, however, the study does not look at the common practices and regulation of hospital hygiene in the setting mentioned above since the paper's aim was to identify the microbes present in hospitals and their susceptibility to antibiotics commonly prescribed in Bangladesh.

The study concludes that microbes causing HAI are commonly found in the environment of healthcare infrastructure in Bangladesh. These microbes also show different levels of susceptibility to antibiotics commonly used in Bangladesh. The common microbes identified are Gram positive *Streptococcus*, *S. aureas*, coagulase negative *Staphylococcus*, Gram negative *Pseudomonas* and *Serratia*. Almost all of these microbes show resistance to cefipime, a fourth generation of cephalosporin. However, ciprofloxacin and vancomycin shows to be most effective against the above identified bacteria. The study has also identified 'satellite colonies' inside the zone of inhibition of some antibiotic susceptible bacteria.

COMPETING INTEREST

The authors declare that they have no competing interests.

AUTHORS CONTRIBUTION

Conceived and designed the experiments: NI & NM Performed the experiments: NM & NI, Data analysis: NM Wrote the paper: NI, NM, AKM Moniruzzaman Mollah. All authors have read the manuscript to revise it carefully and have approved the final manuscript.

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20

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21