ISOLATION, IDENTIFICATION AND ANTIMICROBIAL SENSITIVITY PATTERN OF BACTERIAL ISOLATES FROM TRACHEAL ASPIRATE OF ICU PATIENTS OF CENTRAL DHAKA, BANGLADESH

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ABSTRACT

The study was based on 100 patients of ICU of central Dhaka city, Bangladesh admitted with different admission causes. Data of each patient were collected from the admission form. The data possessed details about the patient's history and the problem list. Here in the study patients were dealt with their endotracheal aspirates (ETA). These aspirates were collected from the ICU and were cultured in media (macConkey agar and blood agar) to isolate and identify the pathogenic organisms in them. The nosocomial infection was also taken under consideration. The organism obtained were *Acinetobacter* sp*., Klebsiella* sp*., E. coli* and *Pseudomonas sp.* Whereas *Acinetobacter* sp. was higher in number and also caused the most nosocomial infection among all. It was observed that among all the patients around 30% were infected by nosocomial infection. Finally DST (Disk Sensitivity Test) was done to see the antimicrobial sensitivity pattern with fifteen different antibiotics against each isolated organisms. All the organisms more or less showed both sensitive and resistance pattern but *Acinetobacter sp* was resistant to all the antibiotics except the antibiotic Colistin. Thereby this is very alarming for the ICU patients of Bangladesh in all the hospitals.

KEYWORDS: ICU, Endotracheal Aspirates, Microbes

INTRODUCTION

An intensive care unit (ICU) is a special department of a hospital or health care facility that provides intensive care medicine. Intensive Care Units cater to patients with the most severe and life-threatening illnesses and injuries; that require constant, close monitoring and support from specialist equipment and medication in order to maintain normal bodily functions. They are staffed by highly trained doctors and critical care nurses who specialize in caring for seriously ill patients. Common conditions that are treated within ICU's include those such as trauma, multiple organ failure and sepsis. Patients may be transferred directly to an Intensive Care Unit from *an* emergency department if required, or from a ward if they rapidly deteriorate; or immediately after surgery if the surgery is majorly invasive and the patient is at high risk of complications. *Streptococcus pneumonia* is one of the most important human pathogens. It is a major cause of pneumonia, meningitis, bacteremia, sinusitis, and otitis media, The World Health Organization estimates that ∼1.6 million people, including up to 1 million children aged <5 years, die of IPD every year with developing countries bearing the greatest burden. With the availability of an effective conjugate vaccine IPD (invasive pneumococcal disease) is also the leading cause of death among vaccine-preventable infectious diseases (Austrain 1970). There has been increasing concern regarding the rise of *Acinetobacter* infections in critically ill patients. We extracted information regarding the relative frequency of *Acinetobacter* pneumonia and bacteraemia in intensive-care-unit (ICU) patients and the antimicrobial resistance of *Acinetobacter* isolates from studies identified in electronic databases.

Acinetobacter infections most frequently involve the respiratory tract of intubated patients and *Acinetobacter* pneumonia has been more common in critically ill patients in Asian (range 4–44%) and European (0–35%) hospitals than in United States hospitals (6–11%). A higher proportion of *Acinetobacter* isolates were resistant to aminoglycosides and piperacillin/tazobactam in Asian and European countries than in the United States. The data suggest that *Acinetobacter* infections are a growing threat affecting a considerable proportion of critically ill patients, especially in Asia and Europe. *Acinetobacter* infections have increased and gained attention because of the organism's prolonged environmental survival and propensity to develop antimicrobial drug resistance (Ashworth *et al* 2004). Studies using tracheal aspirate (TA) have increased our knowledge of the underlying airway inflammation present in the childhood disorders of asthma, cystic fibrosis (CF) and neonatal chronic lung disease (CLD).

A BAL sample is obtained by wedging a bronchoscope or catheter into a bronchus and isolating the distal airway. A volume of saline is instilled and the fluid aspirated back from the airway, using gentle suction. The aim of this process is to obtain aspirated fluid that will contain the microbes, cells, and non-cellular constituents present in the epithelial lining fluid of the small airways and alveoli. Endotracheal suction aspirates (ETSA) are routinely tested as part of the evaluation of febrile episodes in mechanically ventilated patients. In some institutions, ETSA are used as surveillance cultures for nosocomial pneumonia. It is assumed that because aspiration bypasses the oropharynx, ETSA specimens are of higher quality than expectorated specimens (Dorca *et al* 1995). Transthoracic needle aspiration has the potential to improve the diagnostic yield of pneumococcal pneumonia, especially in individuals with large peripheral lesions, including in children who may not produce sputum (Dorca *et al* 1995, Ashworth *et al* 2004). This is a relatively safe procedure if it is performed by experienced staff, but it has yet to be widely adopted because of its invasive nature and concerns about complications. The less compliant lungs of older adults are more prone to pneumothoraces after the procedure, and patients receiving anticoagulation may bleed. The present study was undertaken to isolate and study the frequency of bacterial pathogens from tracheal aspirate; and also to determine the antimicrobial sensitivity pattern of these isolates.

MATERIALS AND METHODS

This was a cross sectional study. This project was carried during a period of 5 months from May to September 2012 in the department of microbiology, ISLAMIC BANK CENTRAL HOSPITAL, KAKRAIL, BANGLADESH (IBCHK). Patient admitted to (IBCHK) ICU and CCU, who were on mechanical ventilation, were included in this study. From the ICU, 100 tracheal aspirates were studied. The following data were collected from the patients enrolled in the study: name, age, gender, underlying illness, date of admission, date of endotracheal insertion, date of endotracheal aspirate collection, duration of hospitalization, duration of mechanical ventilation, date of reintubation, duration of and details of prior antibiotic therapy, clinical diagnosis, and X-Ray report. Other relevant data were recorded from microbiological studies in laboratory. ICU doctor collected ETA (endotracheal aspirate) every time. Hand was washed aseptically and sterile gloves were used in each sample collection.

ETA was collected using suction catheter. It was gently introduced through endotracheal tube for a distance of approx 25-26cm. Gentle aspiration was performed without instilling saline. If Tracheal aspirate was very thick then 2ml of sterile 0.9% normal saline was added to flush the exudates into a container for collection. After collection of endotracheal aspirate from patients in a sterile tube, samples were immediately (within 1 hours) transferred to the microbiology laboratory for Gram staining and culture. Samples were mechanically liquefied and homogenized and then gram stain and culture examination was performed. Using an inoculating loop transfer specimens to glass slides and prepared smears directly from the most purulent portion of the specimen. The Smears were Gram stained and examined. The smears were examined at low power field (HPF) under oil immersion (magnification $\times$ 100) for the quantity of squamous epithelial cells

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(SEC) and polymorphonuclear neutrophils (PMN) and at high-power field (HPF) under oil immersion (magnification ×1,000) for the presence of bacteria.

Semi-quantitative culture was performed based on the four quadrant streak technique using a calibrated loop. At first sample was taken by inoculating loop and a well was made on the agar plate, then four quadrant were made by streaking from well. Each specimen was streaked onto MacConkey agar and 5% sheep blood agar. Plates were inoculated at 37°C for 24 hours. After 24 hours ETA cultures were read semi-quantitatively by observing the growth in the four quadrants, which suggested the approximate number of colony forming units per ml (CFU/ml) of the bacteria in the specimen. The cultures were graded as 1+, 2+, 3+ and 4+. Cultures showing a moderate to heavy growth with 3+ or 4+ grades were considered as positive. The growth of the organisms on culture plates was recorded as rare (≤10 colonies in the first quadrant) or as 1+to 4+ (>10 colonies in the first quadrant to >10 colonies in the fourth quadrant). The following organisms were considered expected respiratory flora (ERF) when they were present in mixed culture.

Extensive biochemical tests were performed in order to measure the viability of biochemical behavior among the strains. According to the manual of methods for general bacteriology by American society of Microbiology, several biochemical tests (Triple Sugar Iron (TSI) Test, Citrate Utilization Test, Motility Indole Urease (MIU) Test, Oxidase Test, Catalase Test, Coagulase Test) were performed to identify bacteria of interest. After isolation and identification, sensitivity of selected organisms against different antibiotics was studied. The organism obtained was diluted in 2-3 ml saline water and then using a cotton swab organism was swabbed on medium, then antibiotic discs were placed on the medium. 25 antibiotic discs were used, among them 15 were used for gram negative organisms and 10 were used for gram positive organisms. , Amikacin, Cefotaxime, Ceftriaxone, Ceftazidime, Cotrimoxazole, Ciprofloxacin, Colistin, Imipenem, Gentamycin, Netilmicin, Tetracycline, Tazobactum , levofloxacin, piperecillin, tobramycin, maropariam were used for the gram negative bacteria.

RESULTS AND DISCUSSIONS

During the five month of study periods, 100 tracheal aspirates from 100 ICU, CCU admitted patients were enrolled. Among them 60 were male and 40 were female. Their average age was between 40-60 years, Of the 100 culture samples 4 types of organisms were isolated from blood culture. Among them most common pathogen was *Acinetobacter* spp. (25%). The other organisms were *Pseudomonas* spp (15%) *Klebsiella* spp (11%), *E. coli* (14%). Here for 7 organisms15 types of antibiotics disks were uses for sensitivity. Among them 14 were used for gram negative organisms. Here most of the antibiotics were resistance except colistin which is normally does not use.

Colistin have shown 100% sensitivity to all organisms. Colistin is normally uses for diabetic patients. Higher percentage is found in the patients of age between 60 to 80. The least number of patients belong to the ages of 30 to 40 (Figure 1). Of all the patients belong to the ages of 40 to 60. Generally most of the patients are admitted with diseases like pulmonary attack, stroke, kidney failure or asthmatic patient. The number of patients of both male and female are equal between the ages of 60 to 70. Among all the patients males are found to be higher in number.

Females are found to be higher in number between the ages of 40 to 60 (Figure 2). From the findings it was observed that the antibiotic Cefotaxime and ceftaxidime is most resistant to the organism *klebsiella sp.* Colistin is 100 percent sensitive (Figure 3). In average all the antibiotics are sensitive for the infected patients with *pseudomonas sp* except cotrim which is almost 100 percent resistant. The antibiotics colistin, pipercillin, ciprofloxacin and meropaniam has good sensitivity pattern (Figure 4). In case of the patients infected by the organism *E. coli sp* three are still few antibiotics that are sensitive. Netilmycin, Meropaniam, Piperecillin and Colistin are the most sensitive one and yet can be used

(Figure 5). Among 14 antibiotics 13 are resistant. This is very alarming for the patients infected with *Acinetobacter sp.* Colistin is the only antibiotic that is almost 100 percent sensitive. ceftriaxone and amikacin are 100 percent resistant. Patients infected by these organisms are very vulnerable (Figure 6). This study was on 100 ICU patients; among these patients around 45 patients developed pulmonary disease.

CONCLUSIONS

In our analysis it was found that patients who develop pneumonia their average age is 40-60 years, Gram negative bacteria, *Acinetobacter* sp. Among all the patients, 30% developed nosocomial infection. This is very alarming for developing countries like Bangladesh. The antibiotic Colistin was found sensitive for all the organisms in present study.

Beside Colistin other antibiotics like ciprofloxacin, maropaniam and pipercilin are also sensitive. In case of *Acinetobacter* sp it is very alarming because almost 99 percent of the antibiotic used were resistant. This is may be because of the regular use of these antibiotics due to the frequent nosocomial infection reducing the level of nosocomial infection will also reduce the resistance pattern of these antibiotics.

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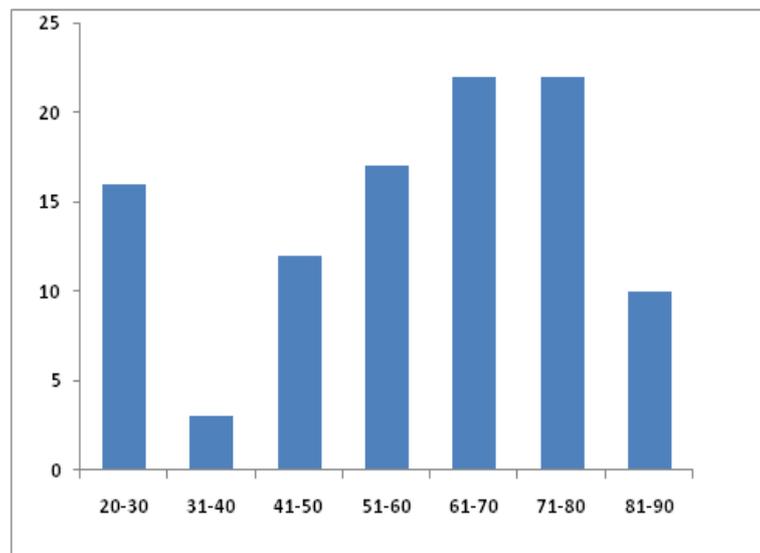
APPENDICES

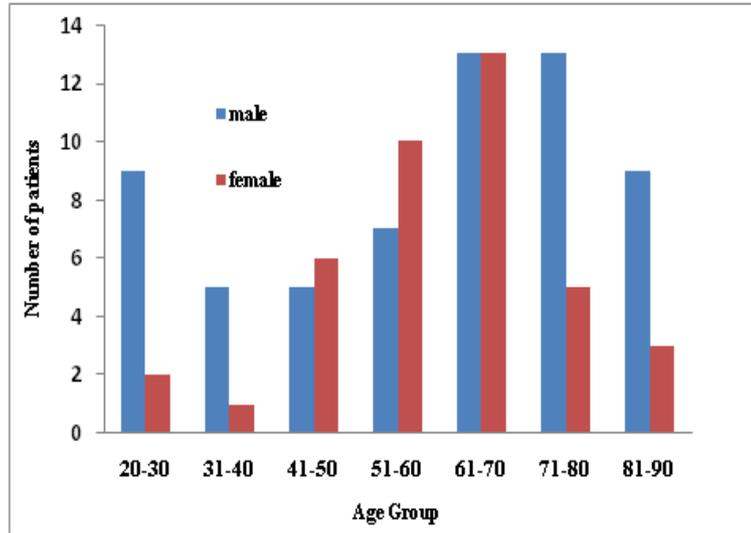**Figure 1: Percentage of ICU Patients of Different Age Group**

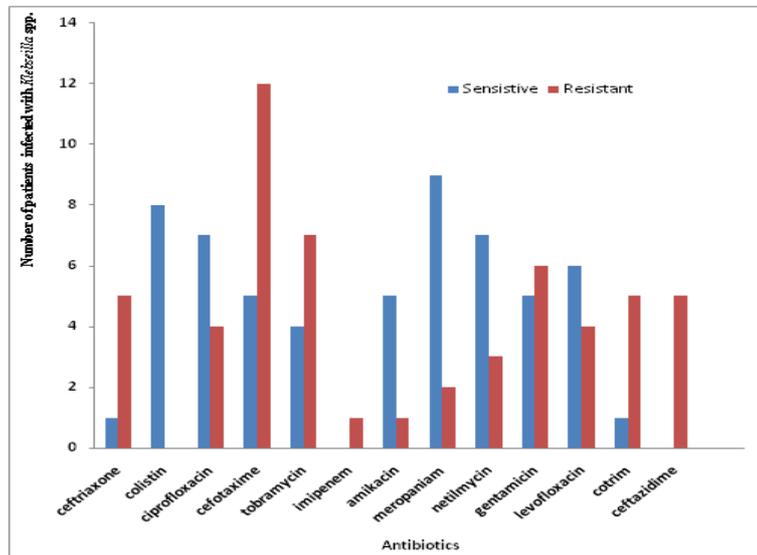**Figure 2: Gender Comparison of ISU Patients**

Figure 3: Patients Infected by *Klebsiella* sp and its Antibiotic Sensitivity Pattern
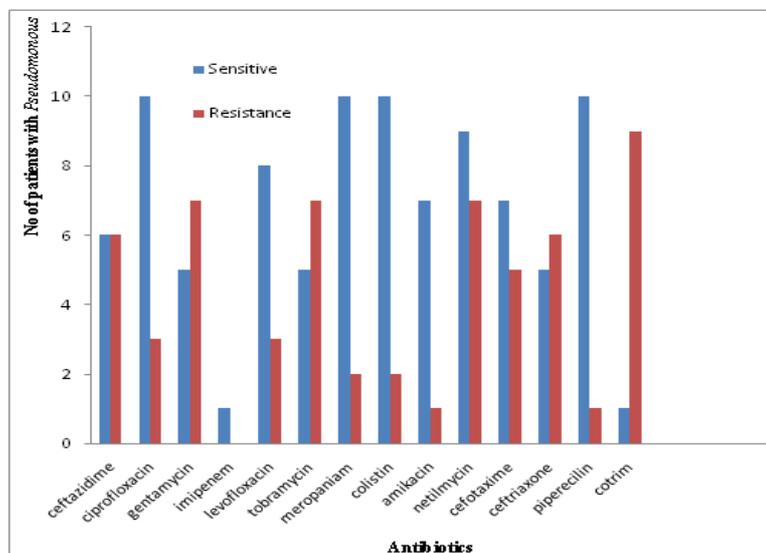

Figure 4: Patients Infected by *Pseudomonas sp* and its Antibiotic Sensitivity Pattern
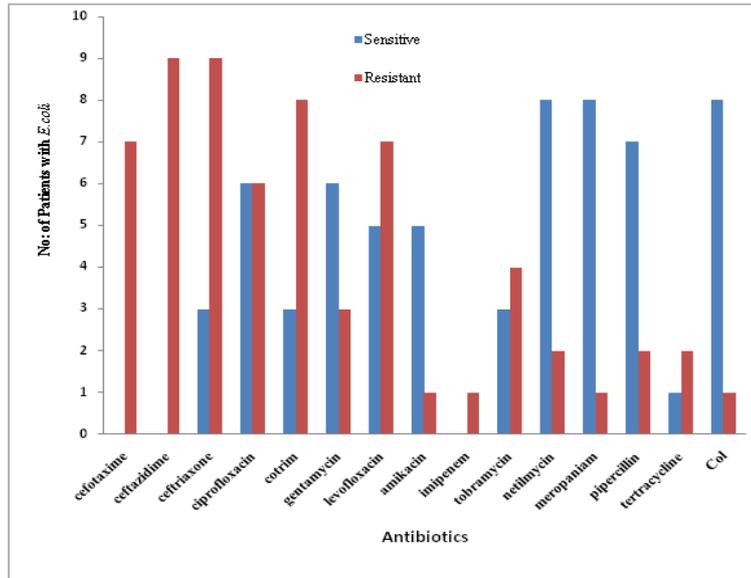
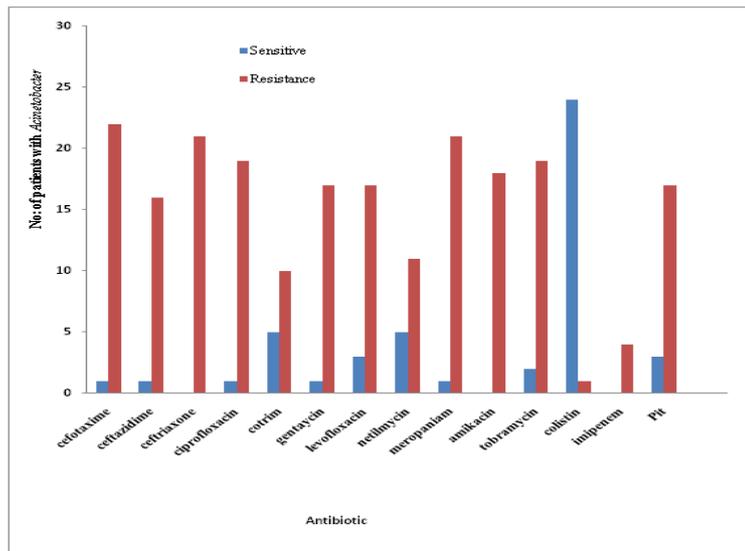**Figure 5: Patients Infected by *E. Coli* sp. and its Antibiotic Sensitivity Pattern**

Figure 6: Patients Infected by *Acinetobacter* sp and its Antibiotic Sensitivity Pattern