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ISOLATION AND IDENTIFICATION OF DIFFERENT BACTERIA FROM DIFFERENT TYPES OF BURN WOUND INFECTIONS AND STUDY THEIR ANTIMICROBIAL SENSITIVITY PATTERN

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ABSTRACT

Out of 150 burn wound swabs samples taken from hospitalized patients in city hospital, Bangladesh patients, 100 samples were found positive by bacterial infection who presented invasive burn wound infection from both sex and average age of 10-55 years. *Pseudomonas aeruginosa* was found to be the most common isolate (23.33%) followed by *Staphylococcus aureus* (15.33%), *Enterobacter* spp. (8.66%), *Proteus vulgaris* (8%), *Micrococcus* sp. (3.33%), *E. coli* (4.66%) and *Klebsiella spp*. (3.33%). Among 8 antibiotics, antibiotic sensitivity pattern of Ciprofloxacin was found to be the most effective drug against most of the Gram-negative and Gram-positive isolates followed by Amikacin, while Chloramphenicol, Doxycycline and Gentamicin were less sensitive to few isolates.

KEYWORDS: Microbes, Antibiotic Sensitivity, Burn Wound

INTRODUCTION

Infection is an important cause of morbidity and mortality in hospitalized burn patients [1], in patients with burn over more than 40% of the total body surface area, 75% of all deaths following thermal injuries are related to infections [2]. The rate of nosocomial infections is higher in burn patients due to various factors like nature of burn injury itself, immunocompromised status of the patient [3], age of the patient, extent of injury, and depth of burn in combination with microbial factors such as type and number of organisms, enzyme and toxin production, colonization of the burn wound site, systemic dissemination of the colonizing organisms [4]. Moreover the larger area of tissue is exposed for a longer time that renders patients prone to invasive bacterial sepsis. In extensive burns when the organisms proliferate in the eschar, and when the density exceeds 100,000 organisms per gram of tissues, they spread to the blood and cause a lethal bacteremia. Therapy of burn wound infections is therefore aimed at keeping the organisms burden below 100,000 per gram of tissues which increases the chances of successful skin grafting.

The denatured protein of the burn eschar provides nutrition for the organisms. Avascularity of the burned tissue places the organisms beyond the reach of host defense mechanisms and systemically administered antibiotics [5]. In addition, cross-infection results between different burn patients due to overcrowding in burn wards [6]. Also thermal destruction of the skin barrier and concomitant depression of local and systemic host cellular and humeral immune responses are pivotal factors contributing to infectious complication in patients with severe burn [7]. Burn wound infections are largely hospital acquired and the infecting pathogens differ from one hospital to another [8]. The burn wound represents a susceptible site for opportunistic colonization by organisms of endogenous and exogenous origin; thermal injury destroys the skin barrier that normally prevents invasion by microorganisms. This makes the burn wound the most frequent origin of sepsis in these patients [9]. Burn wound surfaces are sterile immediately following thermal injury, these wounds eventually become colonized with microorganisms [10], gram-positive bacteria that survive the thermal insult, such as *S. aureus* located deep within sweat glands and hair follicles, heavily colonize the burn wound surface within first 48 h [10]. Topical antimicrobials decrease microbial overgrowth but seldom prevent further colonization with other potentially invasive bacteria and fungi. Gastrointestinal and upper respiratory tract and the hospital environment [11]. Following colonization, these organisms start penetrating the viable tissue depending on their invasive capacity, local wound factors and the degree of the patient s immunosuppression [12]. If sub-eschar tissue is invaded, disseminated infection is likely to occur, and the causative infective microorganisms in any burn facility change with time [13]. Individual organisms are brought into the burns ward on the wounds of new patients. These organisms then persist in the resident flora of the burn treatment facility for a variable period of time, only to be replaced by newly arriving microorganisms. Introduction of new topical agents and systemic antibiotics influence the flora of the wound [14]. The aim of the present study was to obtain information about the type of isolates, identification and antimicrobial sensitivity of bacterial wound infections in burn patients.

MATERIALS AND METHODS

Cultural Media Media used for bacterial isolation and identification are ordinary media such as Blood agar, Nutrient agar, Triptic Soya agar, and special media pseudomonas agar, Salmonella- Shigella agar. MacConkey agar, Mannitol salt agar and Eosin methylene blue agar.

Sample Collection and Inoculate 150 burn wound swabs were taken from burned patients, who presented invasive burn wound infection, from both sex, and average age 10-55 year, admitted to burn unit of teaching medical center of City Hospital, Mohammadpur, Dhaka 1207, Bangladesh , October 2012 to May 2013. The most preferred areas were the upper and lower extremities. The specimens were transported in sterile, leak- proof container to zoonotic diseases unit. All specimens were inoculated on 5% blood agar, MacConkey agar and Chocolate agar plates and incubated overnight at 37 ˚C aerobically. The sample was also put into liquid media (Brain Heart Infusion broth) and was subcultured after overnight incubation onto Blood agar and MacConkey agar. Bacterial pathogens were identified by conventional biochemical methods according to standard microbiological techniques [13]. Antimicrobial susceptibility was performed on Mueller- Hinton agar by the standard disk diffusion method [15]. The antibiotics tested for bacterial isolates were: Ciprofloxacin (Cip5), Amikacin (AK30), Chloramphenicol (C30), Tetracycline (T30), Oxacillin (OX1), Gentamicin (CN 10) and Doxycycline (Do30). The zones of inhibition of bacterial isolates for individual antibiotics were measured in mm by applying ordinary ruler.

RESULTS AND DISCUSSIONS

The prevalence of bacteria in 150 burn wound swabs were shown in the bacterial isolates were found in 100 (66.66%) wound swabs, and only 50 samples (33.33%) were negative in bacterial growth. The results showed that *P. aeruginosa* was the commonest isolate (35 isolates; 23.33%) followed by *S. aureus* (15.33%), *Enterobacter* spp (8.66%), *P. vulgaris* (8 %) *Corynebacterium* spp. *E. coli* (4.66 %) And both *Micrococcus* spp and *Klebsiella* spp., (3.33 %). Most of the isolates showed mixed infection as showed the following results Figure 1, and the antibiotic sensitivity pattern of different bacteria isolated from wound infection shown in the figure 2, as follow the *P. aeruginosa* isolates were moderately resistant to ciprofloxacin (52.17%), and (39.83%) resistant to Amikacin, whereas the resistance was more marked with other antimicrobials like Doxycycline (78.3%), tetracycline (65.57%), and Gentamicin (53.6%). On the other hand, *S. aureus* was resistant 100% to Amikacin, and Gentamicin. The resistance was 72%, 78.8% and 87.7% to Doxycycline, Oxacillin and Tetracycline, respectively. The less resistance was showed by Chloramphenicol (23.57%)

followed by Ciprofloxacin (39.66%). *Klebsiella* spp. were resistant to all of the antibiotics used except Ciprofloxacin (100% sensitive), while *E. coli* was sensitive 100% to both Ciprofloxacin and Chloramphenicol but resistant to the others. *Enterobacter* spp. were resistant to most antibiotics, but were moderately sensitive (50%) to Ciprofloxacin, tetracycline and Doxycycline. *P. vulgaris* also was resistant to 4 antibiotics and showed lower resistance (29.33%) to Chloramphenicol and Gentamicin, but was sensitive to both Ciprofloxacin and Amikacin. *Micrococcus* also was resistant to 5 antibiotics and showed lower resistance (31.66%) to Chloramphenicol and Amikacin, but was sensitive to both Ciprofloxacin and Gentamicin.

Bacteria isolated from only 100 burn wound swabs from the total 150 swab indicated that 66.67% of examined burn patients had invasive burn wound infections, this idea supported the investigation of Moonery *et al.* [9] who explained that the burn wound infections are one of the most important and potentially serious complications that occur in the acute period following injury, also Raja and Singha [16] demonstrated that the infectious complications are considered a major causes of morbidity and mortality and the type and amount of microorganisms on and in the injured tissues influence wound healing. Most of the isolates in our research had mixed with other bacterial species and some of these have shown to be resistant to many antimicrobials, and this indicates the high contamination of burn wounds in our hospitals. In the present study, the most commonly isolated organisms from burned patients were *P. aeruginosa* followed by *S. aureus,* and *Enterobacter* spp. The reasons for this high prevalence may be due to factors associated with the acquisition of nosocomial pathogens in patients with recurrent or long-term hospitalization, complicating illnesses, prior administration of antimicrobial agents, or the immunosuppressive effects of burn trauma.

This evidence was consistent with previous observation mentioned by some workers. Initially, the immunologic response to severe burn injury is proinflammatory but later becomes predominately anti-inflammatory responses in an effort to maintain homeostasis and restore normal physiology; cytokines and cellular response mediate both of these phases [17]. Systemic responses to burn occur by proinflammatory cytokines [18], but the anti-inflammatory responses and the subsequent immunosuppression following burn injury are characterized by a set opposing cells and cytokines, the production and release of monocytes macrophages are decreased following burn injury and sepsis [19], also Embile *et al.* [20] mentioned that the nosocomial transmission of microorganisms to the burn wound occurred by transfer from the hands of health care personnel and through immersion hydrotherapy treatment. Our results of bacterial isolation from burn wound were in accordance with other previous studies. Manjula *et al.* [21] reported that *Pseudomonas* species was the commonest pathogen isolated (23.33 %) from burn wound followed by *S. aureus* (15.33 %), *Klebsiella* spp. (3.33 %) and *Proteus* species (8 %). Arslan *et al.* [23] reported that *Enterobacter* spp. is the main isolate (8.66 %) from burn wound sample, *Micrococcus* spp (3.33%) and *E. coli* (4.66 %). Microbial infection is one of the major serious complications in wound patients, the results of the present study showed that 35 (23.33%) burn wound swabs revealed *P. aeruginosa,* this goes to confirm that *P. aeruginosa* is a major factor in the etiology of wound infection [24], [25]. Our results showed that the rate of isolation of gram-negative organism was more than gram-positive, these results are consistent with those reported by Kehinde *et al.* [26], who reported that the rate of gramnegative bacterial isolation from burn wound was more than twice that gram- positive and they noticed that *Klebsiella* spp. was the pathogen less isolated constituting 3.33% followed by *P. aeruginosa* (23.33 %) and *S. aureus* (15.33%).

The change in the pattern of bacterial resistance in the burn unit is important both for clinical settings and epidemiological purposes. The results of antimicrobial sensitivity showed that *S. aureus* was highly resistant for most of the antibiotics tested, while it had less resistance to ciprofloxacin. The adaptation of *S. aureus* to the modern hospital environment has been marked by the acquisition of drug resistance genes soon after antibiotic introduction [27]. Also the present study showed that *P. aeruginosa* and all other bacterial isolates were highly sensitive to ciprofloxacin while *P. aeruginosa*, *Enterobacter* spp. and *E. coli* were found to be highly resistant to gentamicin, oxacillin and ticarcilin, these results were consistent with investigation of Kehinde *et al.* [26] who reported that more than 72% of the Gram-negative isolates of burn wound were resistant to gentamicin, a commonly used antibiotic for Grampositive infections. Increasing resistance to various anti- Pseudomonas agents has been reported worldwide and this poses a serious problem in therapeutic management of *P. aeruginosa* infections [28]. Also our results explained that most of the isolates were resistant to many antibiotics. Antimicrobial resistance among nosocomial pathogens is a significant problem in clinical settings that may be added to the cost of medical care and the morbidity and mortality of patients [29]. Gram-negative bacteria produce large quantities of type 1 cephalosporinase when exposed to first- generation cephalosporins, ampicillin, and penicillin G, these antimicrobials are readily hydrolysed by this enzyme, and inducible organisms are intrinsically resistant to these agents [29].

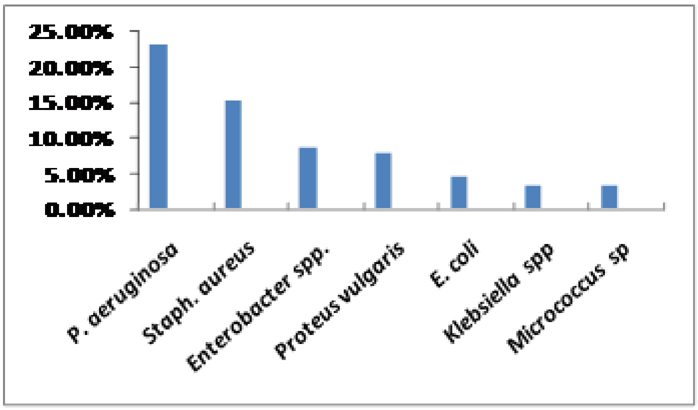
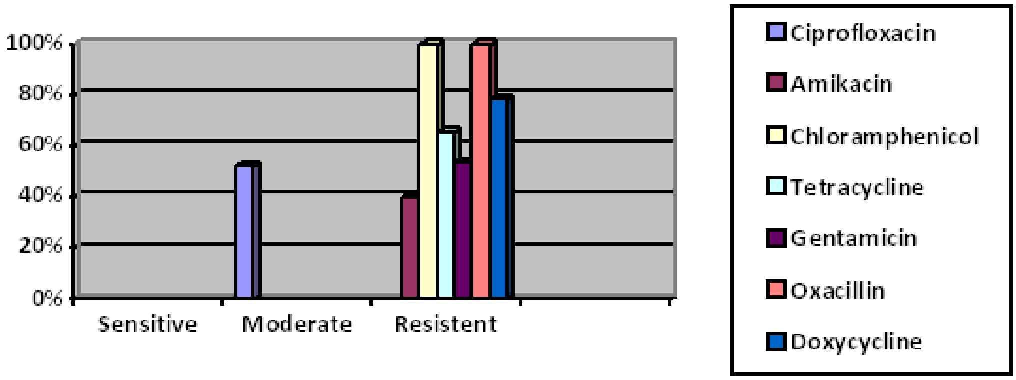
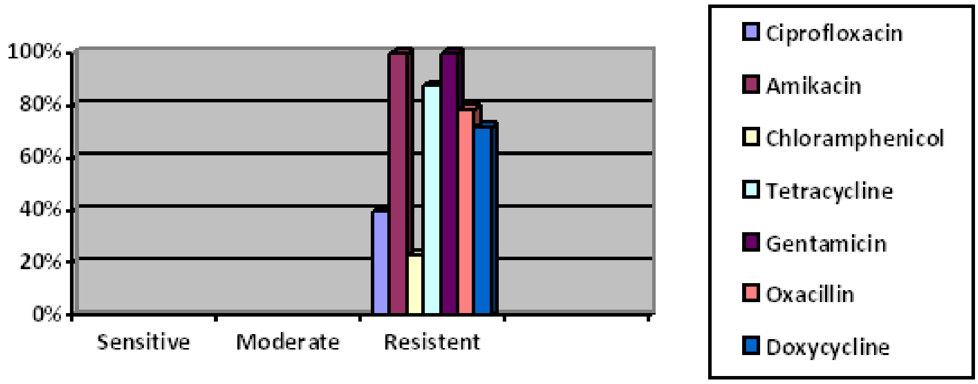


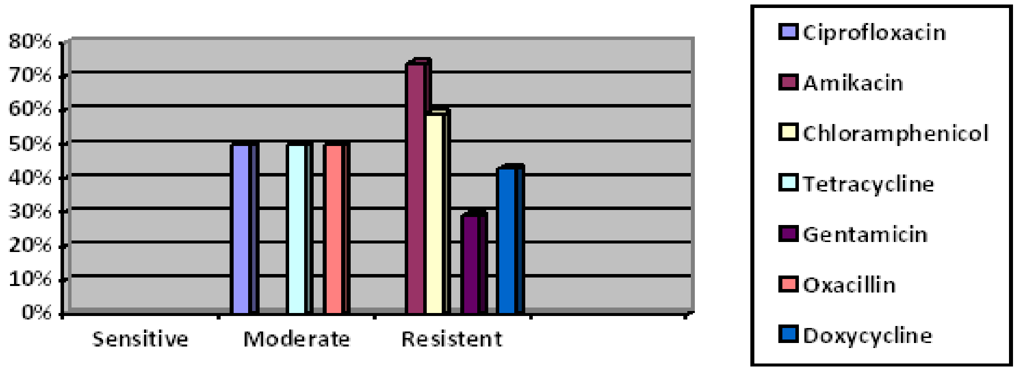
Figure 1: Prevalence of Different Bacteria in Burn Wound Infection



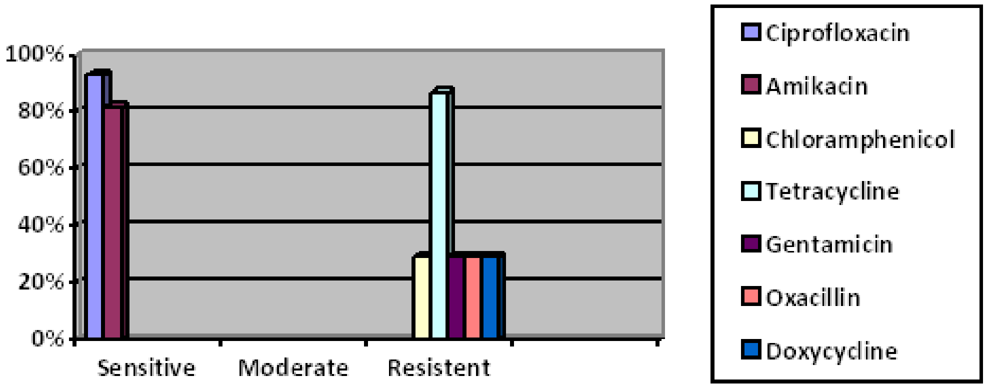
(a) *Pseudomonas auruginosa*



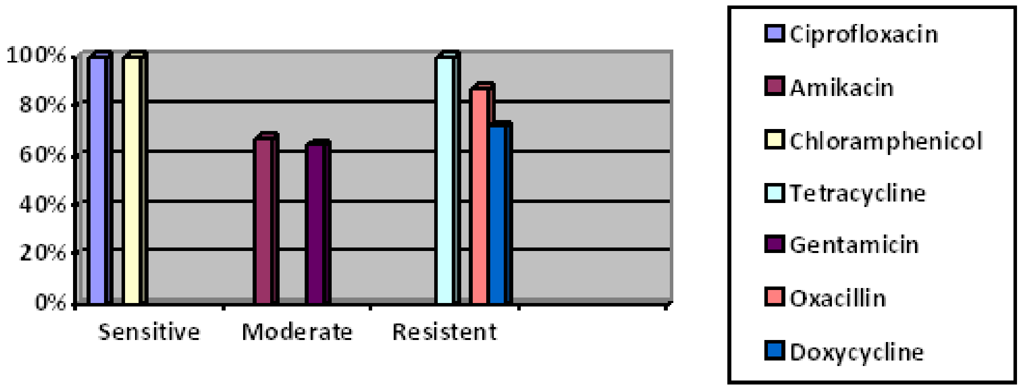
(b) *Staph Aureus*



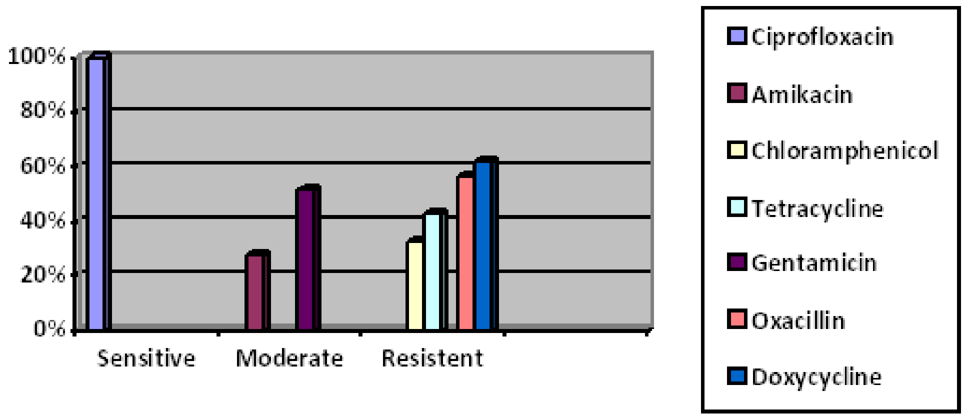
(c). ***Enterobacter*** **spp**



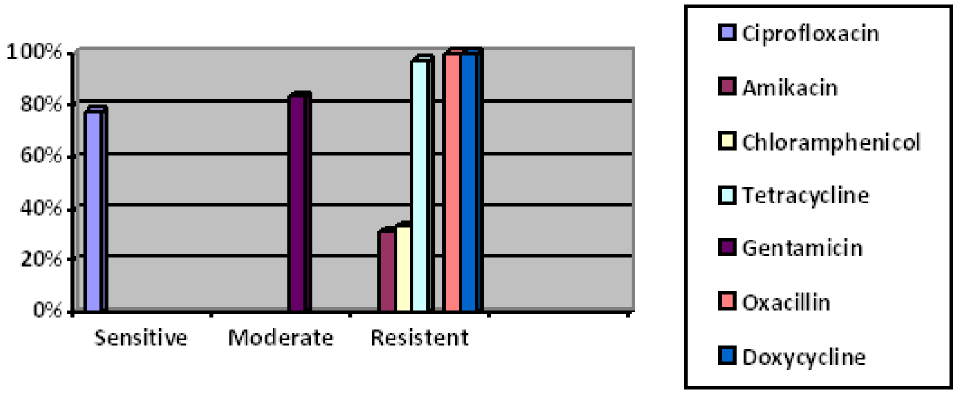
(d). ***Proteus vulgaris***



(e). ***E. Coli***



(f). ***Klesiella*** **spp**



(g). ***Micrococcus*** **sp**

Figuer 2: Antibiotic Sensitivity Pattern of Different Bacteria Isolated from Wound Infection

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