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journal homepage: www.elsevier.com/locate/burns

The analysis of distribution of multidrug resistant *Pseudomonas* and *Bacillus* species from burn patients and burn ward environment

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ARTICLE INFO

Article history:
Accepted 9 October 2014

Keywords:
Multidrug resistant bacteria
B. cereus
P. aeruginosa
Burn patients
Burn ward environment

ABSTRACT

Introduction: Infections caused by multidrug resistant bacteria act as a risk factor for mortality in burns patients. So keeping in view the crucial importance of reliable therapeutic decisions of multidrug resistance bacteria and role of hospital environment in bacteria colonization, our study is based on the evaluation of distribution of *Pseudomonas* sp. and *Bacillus* sp. in burn patients and burn ward environment.

Methods: The present prospective analysis was conducted on the patients undergoing treatment in the Burn ward of Pt. B.D. Sharma University of Health Sciences, Rohtak, Haryana, during the period of January 2012 to March 2013. The multidrug resistant bacteria were characterized by following the CLSI guidelines. Molecular identification isolates were done by amplifying and sequencing 16S rDNA.

Results: In our study out of 510 samples of 280 burn patients, 263 samples were observed sterile and bacterial isolates were obtained from 247 samples. In burn patients out of 247 samples 43 MDR strains, and in burn ward out of 60 samples 4 MDR strain were observed. After 16S rDNA amplification of MDR isolates the prevalent bacterium was belonged to the genus *Bacillus* (8 species; 26 isolates) followed by genus *Pseudomonas* (5 species; 17 isolates). The burn ward environment isolates were *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, *Bacillus cereus* and *Acinetobacter baumanii*.

Conclusion: The major finding of our study is the predominance of *B. cereus* followed by *P. aeruginosa* in burn patients of Pt. B.D. Sharma University of Health Sciences, Rohtak, Haryana. While considering the role of hospital environment, no direct role of environmental isolates was observed in transfer of bacterial infection.

1. Introduction

Burn is one of the most common incidents that create different types of wound infections and act as an important cause of death in patients with burns. In case of severe burns, wound infection becomes critical due to the destruction of skin. As skin act as the first barrier in front of foreign organisms and existence of necrosis, which provides a suitable environment for microbial growth and invasion [1].

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http://dx.doi.org/10.1016/j.burns.2014.10.014

In addition with that burn patients are relatively immunosuppressed caused by the impaired functioning of neutrophils, the cellular and humoral immune system and are at high risk of infections, in particular with nosocomial-acquired multidrug-resistant (MDR) organisms [2,3]. Multi-drug resistant organisms also increase death rates of patients with burn-related sepsis from 42% to 86%. Hospitalized patients in burn care wards are specially at higher risk for hospital-associated infections due to the above mentioned reasons. Organisms associated with nosocomial infections in burn patients include organisms found in the patient's own endogenous (normal) flora or from exogenous sources in the hospital environment [4]. The main exogenous sources that may be transferred to a patient's skin surface via contact with contaminated external environmental surfaces, water, fomites, air, and the soiled hands of health care workers [5]. Numerous reports have demonstrated that hospital environment surfaces are a source of antibiotic-resistant bacteria such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterobacter* sp., *Klebsiella pneumonia*, *Clostridium difficile*, and methicillin-resistant *Staphylococcus aureus* (MRSA). The contamination of wound surfaces by these agents occurs more frequently in developing countries [6,7]. Side by side these drug-resistant bacteria are easily transferred from one patient to another because overcrowding in burn units is another important factor for cross-infection [8].

The organisms that predominate as causative agents of burn wound infection in any burn treatment facility change over time. Gram-positive organisms (methicillin-resistant *S. aureus*), are initially prevalent, then gradually become predominate by Gram-negative opportunists (*P. aeruginosa*, *A. baumannii*, *Enterobacter* sp) that appear to have a greater propensity to invade [9,10]. Out of all MDR bacteria, *P. aerugnosa* (an opportunistic human pathogen) isolated from burn patients proved as most significant bacteria because this bacterium showed best growth on the moist surface of burn wounds and is highly pathogenic in immunocompromised patients [11]. Incidentally *P. aeruginosa* infection is awfully problematic since this organism is inherently resistant to many drug classes and is able to acquire resistance to all effective antimicrobial drugs that make infected burn wounds difficult to treat [12]. Parallel to *Pseudomonas sp.*, our study also highlighted *Bacillus sp.* which has also emerged as one of the new Gram-positive pathogens to cause serious infection in immunocompromised patients. Members of the genus *Bacillus* are aerobic or facultative anaerobic Gram-positive or Gram-variable, spore-forming rods that are ubiquitous in the hospital environment; they may be part of the normal flora, particularly in patients hospitalized for prolonged periods [13]. *Bacillus* causes number of systemic and local infections, including fulminant bacteraemia, central nervous system involvement, catheter related blood stream infection and pneumonia and severe burn wounds [14,15].

The accurate bacterial identification is important in analyzing the epidemiology, antibiotic resistance patterns and outcomes of infections. Traditionally, identification of bacteria in clinical microbiology laboratories was performed using phenotypic tests, including Gram smear and biochemical tests. However, these methods of bacterial identification have major limitations because the organisms with biochemical characteristics that do not fit into the patterns of any known genus and species are difficult to be identified. Second, they cannot be used for uncultivable organisms [16]. Using 16S rDNA sequencing, these problems can be overcome by a single technology, which also facilitates the discovery of novel genera and species. Among the three domains of life, the largest amount of rDNA gene sequencing work concerns bacteria. Using 16S rDNA sequences, numerous bacterial genera and species have been reclassified and renamed; classification of uncultivable bacteria has been made possible [17]. Because of above mentioned reasons for characterization of *Pseudomonas* sp. and *Bacillus* sp. we took advantage of 16S ribosomal DNA (rDNA) sequence data to identify genus- and species- specific 16S rDNA signature sequences of both bacteria's.

So in the nutshell, keeping in view the role of *Pseudomonas* sp. and *Bacillus* sp. (MDR) in immunocompromised burn patients and hospital environment the aim of our study is to evaluate the distribution of multidrug resistant *Pseudomonas* sp. and *Bacillus* sp. in burn patients and burn ward by using 16S rRNA- PCR sequencing tools. In this study an attempt was made to track the path of infection in burn patients i.e. whether the bacterial infection in burn cases come through hospital environment or through cross infection. Because by understanding the routes of colonization one can develop the effective preventive measures against infection.

2. Material and methods

2.1. Study design

The present prospective analysis was conducted on the patients undergoing treatment in the Burn ward of Pt. B.D. Sharma University of Health Sciences, Rohtak, Haryana, during the period of January 2012–March 2013. Bacterial isolates were obtained from the various samples received in Microbiology department of Pt. B.D. Sharma University of Health Sciences, Rohtak, Haryana. The sources of the bacterial isolates were urine, pus, wounds, blood, and body fluids of burn cases. The purity of isolates was determined by macroscopic examination of colonies and microscopic examination of bacteria after Gram staining, and identity of each isolate was confirmed in laboratory by using standard microbiological and other biochemical methods. Bacterial isolates were also grown on selective media like cetrimide agar, *Bacillus cereus* agar base etc. [18,19]. Different isolates were kept in 10% glycerol and frozen at −40 °C for further study. For burn ward environmental sampling various environmental samples (tap water/sink swab/detergents/soap swab/floor/tables/air/hands of nurses and other damp surfaces) were taken once in a week from the various places of burn ward. Environmental samples were taken by using standard protocols like for air sampling we have used sedimentation or depositional method by placing Nutrient agar plates (Simple and inexpensive; best suited for qualitative sampling). Nutrient agar plates were placed in different regions of burn ward keeping in view the temperature, place and humidity [20]. The Inclusion criterion of our study was about 10^8 CFU/ml of bacteria cells were considered as