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Preview Notes

Phenotypic and genotypic aspects of *Pseudomonas aeruginosa* in chronic wounds: a descriptive study

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ABSTRACT

Wound infections can prolong the inflammatory phase of healing. The bacterium *Pseudomonas aeruginosa* is commonly found in chronic wounds and contributes to the chronicity. It also presents natural antimicrobial resistance, which complicates treatment, and may vary depending on the product used in the lesions. **Objective**: To analyze the *Pseudomonas aeruginosa* strains found in the chronic wounds of outpatients treated with carboxymethylcellulose 2% gel or polyurethane board. **Method**: a descriptive study with a quantitative approach, through the collection of biological materials from wounds by swab, culture, identification and molecular characterization of the microorganisms found.

Descriptors: Ulcer; Wound Infection; *Pseudomonas Aeruginosa*; Nursing.

SITUATION AND ITS SIGNIFICANCE

The wound healing process can be accelerated or slowed by factors such as the presence of microorganisms and the products used. Chronic wounds take, on average, fifteen weeks to heal[1] and have a prolonged inflammatory condition[2]. The opportunistic pathogen, *Pseudomonas aeruginosa,* is commonly found in these cases and is difficult to treat due to its natural antimicrobial resistance[2].

Therefore, the relevance of this research is to promote an understanding of the antimicrobial resistance mechanisms of these bacteria to guide specific antibiotic therapies, shorten the treatment of patients, and reduce costs in the health system.

The application of molecular analysis of bacteria is the main factor that justifies this study, given that its unique phenotypic characterization[2] limits the specificity of the microbiological analyses.

HYPOTHESIS

Chronic wounds of outpatients present *Pseudomonas aeruginosa* with different antimicrobial resistance patterns when treated with carboxymethylcellulose 2% gel or with polyurethane plate.

OBJECTIVES

Overall objective: to analyze the *Pseudomonas aeruginosa* strains found in chronic wounds.

Specific objectives: to phenotypically identify strains of *Pseudomonas aeruginosa*; to describe the susceptibility of these to antimicrobial drugs; to detect genes involved in antimicrobial resistance by polymerase chain reaction; to verify genetic diversity by gel-pulsed field electrophoresis; to discuss the influence of the products on the characteristics of the strains found.

METHOD

Descriptive research with a quantitative approach, with data collection performed in the Wound Healing Clinic of Antonio Pedro University Hospital and the Regional Polyclinic of Engenhoca, both in the state of Rio de Janeiro. The population served is approximately 200 patients per year, primarily women over 50 years of age with chronic venous ulcers[3]. The final sample will be determined by convenience, in the form of 70 patients or four months of data collection (November 2014 to February 2015), whichever is achieved first.

Inclusion criteria: aged above 18 years; presenting a chronic wound; using carboxymethylcellulose 2% gel or polyurethane plate in dressing. Exclusion criteria: presenting chronic ulcer fully covered by necrosis; use of immunosuppressive drugs. Discontinuity criterion: switching products during the fifteen days of monitoring.

Two nursing visits (D0 and D15) were conducted, involving the collection of patient identification and clinical data through a specific protocol, for the evaluation of patients with tissue damage. The description of the injury included calculating the area by planimetry, photography and by collecting material in granulation tissue using swab.

Laboratory tests are done at the Faculty of Pharmacy of the UFF / RJ, where the swabs are processed for development in a triphosphate soy broth and agar cetrimide, specific

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for *P. aeruginosa* growth. Suggestive microbial growth will be first submitted to phenotypic identification tests.

The antimicrobial agents used in susceptibility testing by disk diffusion and minimum inhibitory concentration will be determined according to the Clinical and Laboratory Standards Institute (CLSI). The results of these tests will direct the search for specific determinant genes of resistance to antibiotics, using specific primers for polymerase chain reactions (PCR). The amplicons are subjected to electrophoresis on agarose gel 1.5%, impregnated with a solution of ethidium bromide and viewed under ultraviolet light.

The analysis of genetic diversity will be determined by bacterial DNA total fragmentation with the restriction enzymes *SpeI* and by submission to pulsed-field gel electrophoresis (PFGE) using the CHEF-DR III system. The gels are visually inspected under UV light after impregnation with ethidium bromide solution.

Data analysis will be done in two steps. The first will be the evaluation of the clinical data and wounds. The results will be tabulated in spreadsheets using Microsoft Excel software (Serial Number KGFVY-7733B-8WCK9-KTG64-BC7D8). Later it will be assessed for normality through the Shapiro-Wilk test if the final sample is made up of fewer than 50 subjects, or the Kolmogorov-Smirnov test if it's composed of more than 50 participants, and analyzed using descriptive statistics using BioStat 5.3 software (free use license).

The second analysis stage will consist of correlations of the results of the microbiological tests regarding the use of the products. Some of the variables to be used include antimicrobial susceptibility profile and evaluation of genetic determinants of resistance; genetic diversity of *P. aeruginosa* inter and intra-patient; product use time pattern in weeks. These data will also be assessed for normality according to the sample size. The researchers will use Pearson's test (normal data) or Spearman's test (non-normal data), with a 0.05 significance level.

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