ETIOLOGICAL DIAGNOSIS AND PHARMACOTHERAPEUTIC MANAGEMENT OF PARAPNEUMONIC PLEURISY

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Abstract

Pleurisy is an inflammatory process characterized by the accumulation of fluid in the pleural cavity. The most frequent complication of pneumonia is the pleural effusion that can become infected and that needs microbiological analysis of the pleural fluid for an individualized treatment. The aim of this study was to identify the cases of infectious pleurisy by isolating and identifying pathogens in the pleural fluid and determining their antimicrobial susceptibility for a targeted and effective therapy and also to establish antimicrobial resistance phenotypes of the isolated germs. The study was conducted between 04.05.2014 - 01.12.2015 on 150 patients, diagnosed with pleurisy hospitalized in Craiova Emergency County Hospital in in surgical wards: thoracic surgery, general surgery and intensive care. Pathogens were isolated in 43.33% of the pleural fluids. Etiological infective agents palette was diverse, represented by Staphylococcus aureus, methicillin resistant Staphylococcus aureus (MRSA), Streptococcus pyogenes, Enterococcus, Escherichia coli, Klebsiella spp., Pseudomonas spp., non-fermenters Gram negative rods, anaerobic bacteria and Candida albicans. A high proportion of isolated strains showed resistance to chemotherapeutic agents with broad spectrum, which indicates their nosocomial potential. Antimicrobial susceptibility testing is absolutely necessary, in order to choose the most effective therapy.

Rezumat


Keywords: pleurisy, pneumonia, antibiotic resistance

Introduction

Pleurisy is an inflammatory process characterized by the accumulation of fluid in the pleural cavity [10]. The most common cause of pleurisy is parapneumonic pleural effusion characterized by the presence of pneumonia or secondary to an abscess [12]. It was shown that bacterial pneumonias are complicated in 40% cases with parapneumonic...
Materials and Methods

The study was performed between 04.05.2014 - 01.12.2015 on 150 adult patients diagnosed with parapneumonic pleurisy, admitted in the Clinical Emergency County Hospital of Craiova, Romania, in surgical wards and intensive care unit (ICU). The pleural fluid was collected through puncture and it was subjected to classical microbiological diagnosis. From the pleural fluids there were performed two smears coloured with Gram stain (Sanimed, Romania) and May Grunwald-Giemsa (Sanimed, Romania), which were examined with a Nikon Eclipse 8000 optical microscope, for bacterial morphology, layout and presence of epithelial, mesothelial and inflammatory cells. The fluids were cultured on four culture media: Columbia blood agar (Biomerieux, USA) in aerobic and anaerobic atmosphere (obtained with the system GenBag, Biomerieux, USA), MacConkey (Sanimed, Romania), and Sabouraud with chloramphenicol (Sanimed, Romania). The colonies grown after 24 hours of incubation (48 hours for anaerobic plates) were identified by culture properties, by biochemical properties (media MIU, TSI, Chapman, lysin and sodium citrate provided by Sanimed, Romania) and confirmed by latex agglutination for Staphylococcus, Streptococcus and Enterococcus genera.

Antimicrobial testing was performed by disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines 2016, using antibiotic disks provided by Oxoid, USA: Amikacin (AK), Amoxicillin (AX), Ampicillin (AMP), Amoxicillin + clavulanate (AMC), Cefepim (FEP), Cefoxitin (FOX), Cefoperasone - sulbactam (CES), Cefpirom (CPO), Ceftazidime (CAZ), Ceftriaxone (CRO), Cefuroxime (CXM), Cephalotin (CPT), Ciprofloxacin (CIP), Clarithromicin (CLR), Cloramfenicol (C), Gentamycin (CN), Linezolid (LZD), Meropenem (MEM), Norfloxacin (NOR), Oxacillin (OX), Penicillin (P), Piperacillin-tazobactam (TPZ), Rifampin (RA), Teicoplanin (TEC), Tygceclcin (TGC). Each patient included in the study provided a written informed consent. The study was conducted in accordance with the World Medical Association Declaration of Helsinki and approved by the Institutional Ethics Committee of Clinical Emergency Hospital of Craiova, Romania.

Statistical analysis

For storing the antimicrobial resistance data we used Whonet 5.6 software (World Health Organisation). For statistical analyses we used Statistical Package for the Social Sciences (SPSS) 20.0 software (IBM Corporation, New York, USA). The antimicrobial resistance was expressed as the ratio between the number of isolates tested as resistant over the number of isolates tested to the antibiotic. For each isolate, it was calculated the antibiotic resistance index (MAR) as the ratio between the number of antibiotics at which the isolate was resistant over the number of antibiotics tested for that isolate. The correlation between the resistance of the same strain to two different antimicrobial agents was assessed by the kappa agreement coefficient.

Results and Discussion

Pleural infection rates

Microbial pathogens were identified in pleural fluid in 65 patients (43.33%) in singular or multi bacterial infection. 91 bacterial strains were isolated and identified. Analysing the origin of patients by hospital ward, it was found that most of them were hospitalized in the Department of Thoracic Surgery (82.42%), followed by the Intensive Care Unit (13.19%) and other surgical wards (4.40%).

Pleural effusion aetiology

The most frequently isolated species was Pseudomonas aeruginosa (29.31%), followed by anaerobic germs (21.55%), Staphylococcus aureus (17.24%), non-fermenters Gram negative rods (NFR) (14.66%) and in lesser percentages methicillin resistant Staphylococcus aureus (MRSA), Escherichia coli, Streptococcus pyogenes, Enterococcus and Candida albicans (Figure 1).

In 11 cases (7.3%) there were identified combinations of two or three germs of the genera Klebsiella pneumoniae, Pseudomonas aeruginosa, MRSA, E. coli and NFR. Each double association was observed only once and only the triple combination of non-fermenters, Pseudomonas aeruginosa and Klebsiella pneumoniae was found in two patients.
Antimicrobial susceptibility testing

The *Staphylococcus* strains had 100% resistance to: ampicillin, amoxycillin, amoxycillin with clavulanic acid, cefuroxime, cefepime, highly resistant to cefpirome, (90.91%), ceftriaxone (87.50%), ceftazidime (87.50%), penicillin (80.00%). No resistance was found to linezolid, teicoplanin and tazobactam with piperacillin. A low resistance was recorded to amikacin (11.1%) and rifampicin (20.00%). 66.67% resistant to cefoxitin were considered resistant to oxacillin (MRSA) (Figure 2).

Strains of *Klebsiella pneumoniae* were 100% resistant to ampicillin, amoxycillin with clavulanic acid, amoxycillin, ceftazidime, gentamycin, cefpirome, and cefuroxime. A high resistance has been found to penicillin (75.00%), rifampin (75.00%), oxacillin (66.67%), tigecycline (66.67%). All strains were susceptible to meropenem (Figure 3).

The strains of *Pseudomonas* spp. isolated were completely (100%) resistant to ampicillin, amoxycillin, cefpirome, in high percentages to ceftriaxone (91.67%), penicillin (90.00%), cefuroxime (87.50%), ceftazidime (85.00%), amoxycillin with clavulanic acid (75.00%), gentamicin (73.33%) and cefepime (71.43%). Small percentages of resistance were recorded in teicoplanin (9.09%) and linezolid (11.11%) (Figure 4).
Antimicrobial testing of the non-fermenters Gram negative rods showed complete resistance of 100% to ampicillin and amoxicillin. A marked resistance was found to cefuroxime (90.91%), cefpirome (88.89%), amoxycillin with clavulanic acid (75.00%), ceftriaxone (75.00%). A low resistance has been found for tazobactam piperacillin (20.00%), amikacin (25.00%) and tigecycline (33.33%) (Figure 5). No strains were resistant to colistin [15].

From all the strains tested to antimicrobials, *Pseudomonas spp.* had the highest resistance, calculated as antibiotic resistance index (MAR), of 81.45%, followed by non-fermenters Gram negative rods (MAR = 68.92%), and a much lower MAR was observed in *Klebsiella spp.*, *Staphylococcus spp.* and *E. coli* (Table I).

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<tr>
<th>Bacterial species</th>
<th>Antibiotic resistance index</th>
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<tr>
<td><em>Staphylococcus aureus</em></td>
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<td><em>Streptococcus spp.</em></td>
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<td><em>Enterococcus spp.</em></td>
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<td><em>Escherichia coli</em></td>
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<td><em>Klebsiella pneumoniae</em></td>
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<td><em>Pseudomonas spp.</em></td>
<td>81.45%</td>
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<td>Non-fermenters Gram negative rods</td>
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It was performed a statistical analysis in order to correlate resistances of the same strain to different antibiotics. For *Pseudomonas spp.*, it was detected a correlation between resistance to cephalosporins: cefazidime and ceftriaxone, or cefoperasone-sulbactam and cephalotin.

We also observed that for *Staphylococcus aureus* there was a high correlation between oxacillin and amoxicillin resistances and between tygecyclin and teicoplatin resistances.

The long period of pleurisy and pleural fluid infection can be explained by persistent pulmonary infections, which intermittently discharge germs that colonize the pleural fluid [4]. Tuberculosis can be such a condition that leaves sequelae that subsequently can infect, become abscesses and produce pleurisy [18]. In the surgical wards the species with nosocomial potential are *Pseudomonas spp.* and MRSA [1, 17], fact confirmed by the present study. The pleurisy with *Klebsiella spp.* had a prevalence of 7.76%, compared with the prevalence of *Klebsiella spp.* infections in other surgical infections in our hospital of 16.34% [7]. Infections with these strains contributed to
increasing the admission period and in most cases they were resolved after 2 - 3 months of antibiotic treatment in the hospital.

Table II

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In some patients who were hospitalized for a longer period due to the chronic evolution, there were collected several samples of pleural fluid, in which bacteriological analysis revealed the presence of an infectious agent (Staphylococcus aureus) present in the first week of admission, followed by its disappearance as a result of appropriate therapy and the subsequent reinfection of the pleural fluid after three weeks with a multi-resistant hospital germ (Pseudomonas spp.).

In another case, the patient presented from the beginning coinfection of pleural fluid with Pseudomonas spp. and Klebsiella spp. Two weeks later, the infection with Klebsiella spp. resolved, but in the pleural liquid samples persisted Pseudomonas spp., with an episode of transient suprainfection with non-fermenters Gram negative rods present in aeromicroflora (with the same pattern of resistance to antibiotics). Massive and prolonged therapy with antibiotics has encouraged the development of Candida albicans, which was isolated subsequently.

In these cases it was observed the increase in time of the antibiotic resistance of Pseudomonas spp., measured by the antibiotic resistance index, which was previously reported in our hospital [6]. Thus, it was demonstrated that the resistance of Pseudomonas spp. to ceftazidime, ciprofloxacin and gentamicin can develop rapidly after one week [11]. The quinolones are good therapeutic choices, because penetrate well into pleural fluid, though they have kidney toxicity [20, 21], and the resistance was maintained throughout the follow-up. Instead, resistance to meropenem and tigecycline had a wavy character.

These variations can be explained by the genetic adaptation phenomena of Pseudomonas spp. under the selective pressure of the antibiotic [9]. For Pseudomonas spp. it was shown that normally exist drug-resistant populations in small percentage, which can develop under antibiotic selective pressure [3]. For example AmpC gene encodes a beta-lactamase plasmid present in all strains of Pseudomonas spp., which when activated cleaves penicillins and cephalosporins [13]. The wavy behaviour of resistance to meropenem can be explained by the rapid loss of resistance after cessation of therapy with meropenem [16].

The MRSA prevalence in pleural effusions off 11.49% was comparable with other studies [5]. The correlations between antibiotic resistances for Staphylococcus aureus can be explained by the presence of mecA gene which encodes a modified
penicillin binding protein (PBP2A) that provides resistance to penicillins and cephalosporins. The non-fermenters had generally higher resistance rates than other bacteria, especially considering 4th generation cephalosporins, penicillins with inhibitors and carbapenems, due to the natural resistance mechanisms as the lack of porins. The resistance to carbapenems can be correlated with greater consumption of meropenem in our hospital. The anaerobic bacteria often are involved in deep lung abscesses and can reach the pleural through contiguity. They usually respond to metronidazole and to topical treatment (pleural puncture and washing with hydrogen peroxide). Despite the apparently easiness to cure pleurisy with anaerobes, we must ensure the sterilization of the pulmonary site to prevent recurrences. Recently it has been proposed to reduce the standard length of antibiotic therapy in parapneumonic pleurisy, from 14 - 21 days to 8 days. This reduction could prevent the selection of resistant strains without significantly affecting the clinical cure rate. But further studies are needed to demonstrate whether by decreasing the duration of treatment does not increase the risk of recurrence of the infection [19].

Conclusions
Etiological palette of the infective agents isolated and identified from the pleural fluid was diverse. It has been found that a high proportion of bacterial strains showed resistance to chemotherapeutic agents with broad spectrum, which indicates their nosocomial potential. Antimicrobial susceptibility testing is absolutely necessary, in order to choose effective therapy. It is recommended researching and evaluating the effectiveness of strategies to prevent emergence of microbial strains with resistance to antibiotics in patients with parapneumonic pleurisy.

References
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