

ETIOLOGICAL DIAGNOSIS AND PHARMACOTHERAPEUTIC MANAGEMENT OF PARAPNEUMONIC PLEURISY

DANIELA CĂLINA^{1#}, LUCICA ROȘU^{2#}, ALEXANDRA FLORIANA ROȘU^{3#}, GABRIEL IANOȘI^{4#}, SIMONA IANOȘI^{5#}, OVIDIU ZLATIAN^{2#}, RADU MITRUȚ^{6#}, ANCA OANA DOCEA^{7#*}, OTILIA ROGOVEANU^{8#}, PAUL MITRUȚ^{9#}, ALINA CRENGUȚA NICOLAE^{10#}, CRISTINA MANUELA DRĂGOI^{10#}, ELIZA GOFIȚĂ^{7#}

¹Clinical Pharmacy Department, University of Medicine and Pharmacy of Craiova, Craiova, Romania

²Microbiology Department, University of Medicine and Pharmacy of Craiova, Craiova, Romania

³Internal Medicine Department, University of Medicine and Pharmacy of Craiova, Craiova, Romania

⁴Surgery Department, University of Medicine and Pharmacy of Craiova, Craiova, Romania

⁵Dermatology Department, University of Medicine and Pharmacy of Craiova, Craiova, Romania

⁶Pathology Department, University of Medicine and Pharmacy of Craiova, Craiova, Romania

⁷Toxicology Department, University of Medicine and Pharmacy of Craiova, Craiova, Romania

⁸Physiotherapy Department, University of Medicine and Pharmacy of Craiova, Craiova, Romania

⁹Internal Medicine Department, University of Medicine and Pharmacy of Craiova, Craiova, Romania

¹⁰Biochemistry Department, Faculty of Pharmacy, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

*corresponding author: ancadocea@gmail.com

All authors have equal contribution

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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

Pleurezia este un proces inflamator caracterizat de acumularea de lichid la nivelul cavității pleurale. Una dintre cele mai frecvente complicații ale pneumoniei este efuzia pleurală, recomandându-se analiza microbiologică a lichidului pleural pentru aplicarea unui tratament specific. Scopul acestui studiu a fost de a identifica cazurile de pleurezii infecțioase prin izolarea și identificarea patogenilor din lichidul pleural și determinarea susceptibilității antimicrobiene pentru o terapie eficientă și țintită, și de a stabili fenotipurile de rezistență antimicrobiană a germenilor izolați. Studiul a fost efectuat între 04.05.2014 - 01.12.2015 pe 150 pacienți diagnosticați clinic cu pleurezie, internați în Spitalul Clinic Județean de Urgență Craiova în secțiile de chirurgie toracică, chirurgie generală și terapie intensivă. Agenți patogeni au fost izolați din 43,33% din lichidele pleurale. Paleta etiologică a agenților etiologici a fost diversă, reprezentată de *Staphylococcus aureus*, *Staphylococcus aureus* rezistent la metilicilină (MRSA), *Streptococcus pyogenes*, *Enterococcus*, *Escherichia coli*, *Klebsiella spp.*, *Pseudomonas spp.*, bacili Gram negativi non-fermentativi, germeni anaerobi și *Candida albicans*. O mare parte din tulpinile izolate au prezentat rezistență la antibiotic cu spectru larg, ceea ce arată potențialul nosocomial. Antibiotograma este absolut necesară pentru alegerea unei terapii eficiente.

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 para-

pneumonic 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

effusion [8]. The rapid diagnosis of pleurisy is essential to prevent chronic evolution [14], so the microbiological analysis of the pleural fluid is indicated after any surgical manoeuvre or traumatism that can affect the pleura.

There are three main mechanisms of seeding the pathogens in the pleura: direct inoculation, which occurs in surgery, trauma or pleural punctures; the spread from an infectious pneumonic site; propagation of an infection from neighbourhood (oesophageal, mediastinal, liver abscess or subfrenic).

The pneumonia can be complicated with pleurisy in rare cases, if the therapeutic management is incorrectly applied or if the patient is subjected to favouring factors as cold, stress, immunosuppressive diseases and therapies [2].

The aim of this study was to identify the cases of infectious pleurisy, excluding other causes; to isolate and identify the pathogens in the pleural fluid and their antimicrobial susceptibility for a targeted and effective therapy and also to establish antimicrobial resistance phenotypes of isolated germs.

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 (Biomérieux, USA) in aerobic and anaerobic atmosphere (obtained with the system GenBag, Biomérieux, USA), Mac-Conkey (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), Clarithromycin (CLR), Cloramfenicol (C), Gentamycin (CN), Linezolid (LZD), Meropenem (MEM), Norfloxacin (NOR), Oxacillin (OX), Penicillin (P), Piperacillin-tazobactam (TPZ), Rifampin (RA), Teicoplanin (TEC), Tygeciclin (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.

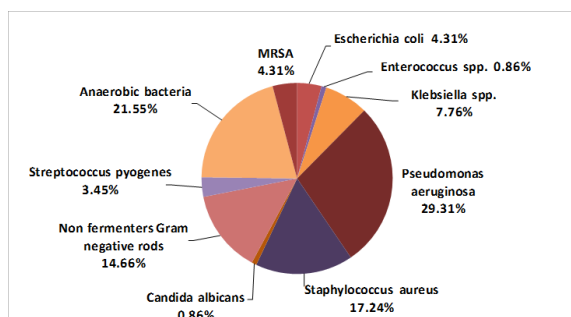


Figure 1.

Microorganisms isolated from the pleural effusions

Antimicrobial susceptibility testing

The *Staphylococcus* strains had 100% resistance to: ampicillin, amoxycillin, amoxycillin with clavulanic acid, cefuroxime, cefepime, highly resistant to ceftazidime (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 2Error! Reference source not found.).

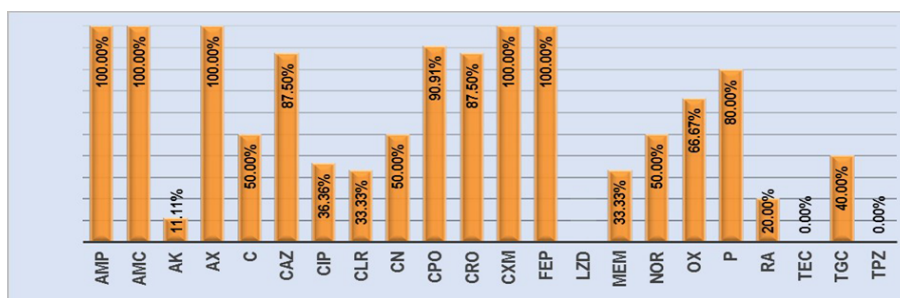


Figure 2.

Antimicrobial resistance profile of *Staphylococcus aureus*

Antibiotic abbreviations: Ampicillin (AMP), Amoxicillin+clavulanate (AMC), Amikacin (AK), Amoxicillin (AX), Chloramphenicol (C), Ceftazidime (CAZ), Ciprofloxacin (CIP), Clarithromycin (CLR), Gentamycin (CN), Ceftazidime (CRO), Cefuroxime (CXM), Cefepime (FEP), Linezolid (LZD), Meropenem (MEM), Norfloxacin (NOR), Oxacillin (OX), Penicillin (P), Rifampin (RA), Teicoplanin (TEC), Tygecyclin (TGC), Piperacillin-tazobactam (TPZ).

Strains of *Klebsiella pneumoniae* were 100% resistant to ampicillin, amoxycillin with clavulanic acid, amoxicillin, ceftazidime, gentamycin, ceftazidime, 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 3Error! Reference source not found.).

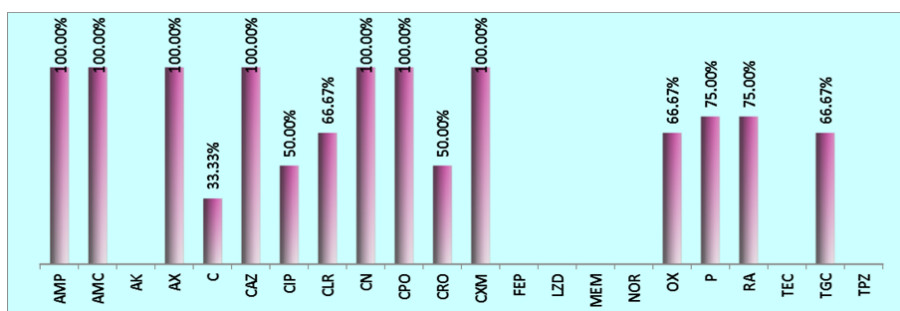


Figure 3.

Antimicrobial resistance profile of *Klebsiella pneumoniae*

The strains of *Pseudomonas spp.* isolated were completely (100%) resistant to ampicillin, amoxicillin, ceftazidime, gentamycin, ceftazidime, 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).

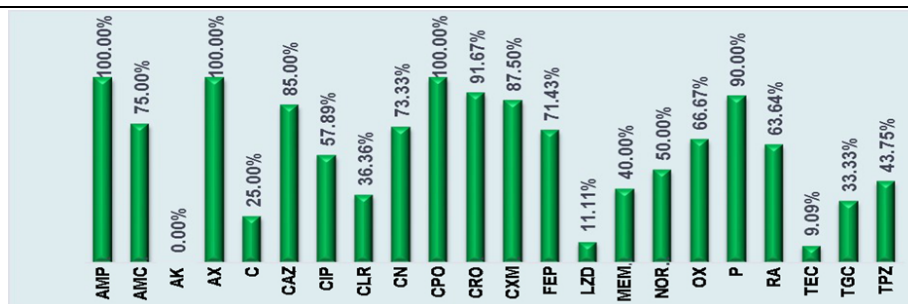


Figure 4.

Antimicrobial resistance profile of *Pseudomonas spp.*

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%), ceftazidime (88.89%), amoxicillin 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].

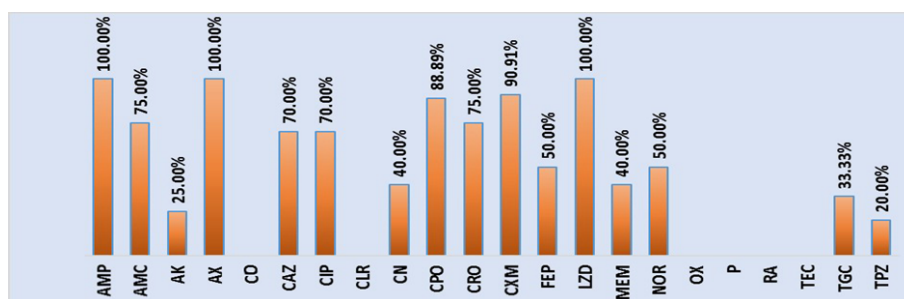


Figure 5.

Antimicrobial resistance profile of non-fermenters Gram negative rods

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).

Table I

Resistance index of the isolated bacterial species

Bacterial species	Antibiotic resistance index
<i>Staphylococcus aureus</i>	46.61%
<i>Streptococcus spp.</i>	42.86%
<i>Enterococcus spp.</i>	42.85%
<i>Escherichia coli</i>	37.22%
<i>Klebsiella pneumoniae</i>	47.14%
<i>Pseudomonas spp.</i>	81.45%
Non-fermenters Gram negative rods	68.92%

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 (Table II) correlation between resistance to cephalosporins: ceftazidime 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 tigecycline and teicoplanin 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

Correlation matrix between antibiotic resistances of *Pseudomonas* spp. expressed by kappa agreement coefficient

Antibi otic	AK	AMC	AMP	CAZ	CES	CIP	CN	CPO	CPT	CRO	CXM	ETP	FEP	MEM	TGC	TPZ
AK	100.0 0%	20.00 %	80.00 %	20.00 %	75.00 %	75.00 %	100.0 0%	28.57 %	100.0 0%	62.50 %	44.44 %	100.0 0%	80.00 %	66.67 %	50.00 %	33.33 %
AMC		100.0 0%	66.67 %	75.00 %	50.00 %	33.33 %	75.00 %	75.00 %	75.00 %	100.0 0%	100.0 0%	100.0 0%	80.00 %	80.00 %	75.00 %	100.0 0%
AMP			100.0 0%	75.00 %	75.00 %	33.33 %	75.00 %	75.00 %	75.00 %	66.67 %	50.00 %	100.0 0%	75.00 %	100.0 0%	66.67 %	75.00 %
CAZ				100.0 0%	50.00 %	40.00 %	40.00 %	60.00 %	50.00 %	71.43 %	83.33 %	50.00 %	40.00 %	50.00 %	80.00 %	60.00 %
CES					100.0 0%	66.67 %	80.00 %	60.00 %	100.0 0%	66.67 %	33.33 %	100.0 0%	100.0 0%	75.00 %	50.00 %	50.00 %
CIP						100.0 0%	60.00 %	100.0 0%	100.0 0%	87.50 %	75.00 %	100.0 0%	100.0 0%	50.00 %	80.00 %	75.00 %
CN							100.0 0%	40.00 %	60.00 %	57.14 %	60.00 %	75.00 %	60.00 %	66.67 %	66.67 %	80.00 %
CPO								100.0 0%	100.0 0%	100.0 0%	80.00 %	100.0 0%	100.0 0%	33.33 %	66.67 %	100.0 0%
CPT									100.0 0%	100.0 0%	66.67 %	100.0 0%	100.0 0%	75.00 %	80.00 %	66.67 %
CRO										100.0 0%	88.89 %	100.0 0%	80.00 %	50.00 %	60.00 %	100.0 0%
CXM											100.0 0%	66.67 %	60.00 %	50.00 %	50.00 %	100.0 0%
ETP												100.0 0%	100.0 0%	75.00 %	75.00 %	75.00 %
FEP													100.0 0%	75.00 %	80.00 %	25.00 %
MEM														100.0 0%	0.00 %	0.00 %
TGC															100.0 0%	80.00 %
TPZ																100.0 0%

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 pleura 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

1. de Abreu P.M., Farias P.G., Paiva G.S., Almeida A.M., Morais P.V., Persistence of microbial communities including *Pseudomonas aeruginosa* in a hospital environment: a potential health hazard. *BMC microbiology*, 2014; 14(1): 118.
2. Ahmed R.A., Marrie T.J., Huang J.Q., Thoracic empyema in patients with community-acquired pneumonia. *The Am. J. Med.*, 2016; 119(10): 877-883.
3. Alonso A., Campanario E., Martínez J.L., Emergence of multidrug-resistant mutants is increased under antibiotic selective pressure in *Pseudomonas aeruginosa*. *Microbiology*, 1999; 145(10): 2857-2862.
4. Arancibia F., Bauer T.T., Ewig S., Mensa J., Gonzalez J., Niederman M.S., Torres M., Community-acquired pneumonia due to gram-negative bacteria and *Pseudomonas aeruginosa*: incidence, risk, and prognosis. *Arch. Intern. Med.*, 2002; 162(16): 1849-1858.
5. Chubar I.V., Analysis of microflora of pleural cavity in pleural empyema. *Klinichna khirurhiia / Ministerstvo okhorony zdorov'ia Ukraïny, Naukove tovarystvo khirurhiv Ukraïny*, 2016; 4: 47-49.
6. Colcea C., Zlatian O.M., Rosu L., Calina D., Rosu A.F., Chiutu L., Docea A.O., Correlation between consumption of antimicrobials in the intensive care unit and resistance of *Pseudomonas* and *Klebsiella* in pulmonary infection. *Eur. J. Hosp. Pharm.*, 2014; 21(A16): 2047.
7. Cristea O.M., Zlatian O.M., Dinescu S.N., Bălăsoiu A.T., Avramescu C., Bălăsoiu M., Niculescu M., Calina D., A comparative study on antibiotic resistance of *Klebsiella* strains from surgical and intensive care wards. *Current health sciences*, 2016; 42(2): 169-179.
8. Hampson C., Lemos J.A., Klein J.S., Diagnosis and management of parapneumonic effusions. *Seminars in Respiratory and Critical Care Medicine*, 2008; 29(4): 414-426.
9. Hancock R.E., Speert D.P., Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and impact on treatment. *Drug resistance updates: reviews and commentaries in antimicrobial and anticancer chemotherapy*, 2000; 3(4): 247-255.
10. Kass S.M., Williams P.M., Reamy B.V., Pleurisy. *Am. Fam. Physician*, 2007; 75(9): 1357-1364.
11. Liapakis I.E., Kottakis I., Tzatzarakis M.N., Tsatsakis A.M., Pitiakoudis M.S., Ypsilantis P., Lightz R.W., Simopoulos C.E., Bouros D.E., Penetration of newer quinolones in the empyema fluid. *Eur. Respir. J.*, 2004; 24(3): 466-470.
12. Light W., Garry Lee Y.C., Textbook of Pleural Diseases, 3rd Edition, CRC Press, New York, USA, 2008; 295-330.
13. Liu Y., Liu X., Detection of AmpC β -lactamases in *Acinetobacter baumannii* in the Xuzhou region and analysis of drug resistance. *Experimental and therapeutic medicine*, 2015; 10(3): 933-936.
14. Muir J.F., Cuvelier A., Molano C., Benhamou D., Diagnosis of pleurisy in an emergency setting. *Rev. Prat.*, 2007; 57(5): 479-488.
15. Neonakis I.K., Samonis G., Messaritakis H., Baritaki S., Georgiladakis A., Maraki S., Spandidos D.A., Resistance status and evolution trends of *Klebsiella pneumoniae* isolates in a university hospital in Greece: ineffectiveness of carbapenems and increasing resistance to colistin. *Chemotherapy*, 2010; 56(6): 448-452.
16. Reinhardt A., Köhler T., Wood P., Rohner P., Dumas J.L., Ricou B., van Delden C., Development and persistence of antimicrobial resistance in *Pseudomonas aeruginosa*: A longitudinal observation in mechanically ventilated patients. *Antimicrob. Agents Chemother.*, 2007; 51(4): 1341-1350.
17. Sax H., Uçkay I., Balmelli C., Bernasconi E., Boubaker K., Mühlemann K., Ruef C., Troillet N., Widmer A., Zanetti G., Pittet D., Overall burden of healthcare-associated infections among surgical patients. Results of a national study. *Ann. Surg.*, 2011; 253(2): 365-370.

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18. Soroceanu V., Rais C., Stefanescu E., Brumărel M., Safta V., Adauji S., Priscu V., Taerel A.E., Epidemiological and economic aspects of tuberculosis in children. A comparative analysis: Romania vs. the Republic of Moldova. *Farmacia*, 2016; 64(1): 152-158.
 19. Staicus C., Calina D., Rosu L., Rosu A.F., Zlatian O., The pharmacotherapeutic management of parapneumonic pleurisy. *Eur. J. Hosp. Pharm.*, 2015; 22: A58-A59.
 20. Stoica M.C., Vari C.E., Imre S., Vancea S., Dogaru M.T., Carașca E., Tarța I.D., Dogaru G., Căldăraru C.D., Correlations between the stages of kidney disease and the pharmacokinetic parameters of orally administered ciprofloxacin at patients with chronic kidney disease. *Farmacia*, 2015; 63(5): 739-744.
 21. Székely-Szentmiklósi B., Tőkés B., Study of ofloxacin - random by methylated - β - cyclodextrin inclusion complex. *Farmacia*, 2016; 64(1): 147-151.