THE UNIVERSITY OF MEDICINE AND PHARMACY

"CAROL DAVILA", BUCHAREST

DOCTORAL SCHOOL

MEDICINE

MEDICO-LEGAL STUDY

OF HEALTHCARE ASSOCIATED INFECTIONS

PHD THESIS SUMMARY

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2023

Content

Introduction

The motivation for choosing the research topic

Worldwide, healthcare-associated infections (HCAIs) are a major public health problem[1], given their impact on in-hospital mortality and morbidity, prolonging hospital stays and increasing healthcare costs, being a burden on healthcare systems worldwide[2]. However, when it comes to assessing the impact of this problem at international level, difficulties arise either due to the underreporting of the HCAIs or the lack of national continuous surveillance systems, currently in place in only 19 European Union states[3].

Surveillance systems are predominantly present in high-income countries showing a prevalence of AIAM here between 3.5% and 12%, while in low- and middle-income countries we have a fragmented picture due to either the lack or inadequate quality of data showing a prevalence ranging between 5.7% and 19.1%[2], [4].

In Europe, average prevalence decreased from 7.1% (2010) to 5.7% (2016-2017)[4], [5]. In the United States, from an average prevalence of 3% (2015)[6], when the national baseline was established, progress on national healthcare-associated infection control and the burden of HCAI is assessed using the standardized infection ratio (SIR), showing an overall decrease in HCAI in all health care settings[6]. AIAMs in Romania are underreported and the burden is greatly underestimated, with hospital reports showing a prevalence rate of 0.2-0.25%[7]with a higher value in a 2017 point prevalence study of 2.6%[8].

Research hypothesis

Given that HCAIs are encountered in medico-legal practice on the one hand when they are involved in the thanatogenerative chain and on the other hand in medico-legal expertise when patients or their families accuse medical facilities or staff of the appearance of the infection and demand financial compensation [9], thus a role of the specialty in this public health problem is taking shape.

Post-mortem examinations play a role in public health, having several epidemiological implications. In the last three decades, systematic surveillance has been attempted in relation to bioterrorism, infectious diseases[10]and drug-related mortality[11], as well as recently for systematic prospective post-mortem surveillance of deaths related to Covid-19[12].

GENERAL PART

Chapter 1. Surveillance of Healthcare Associated Infections

Surveillance in medicine, especially in public health, recognizes case definitions as an important element of surveillance systems, as they can have a crucial impact on system sensitivity and ensure comparability and consistency of collected data.[13].

In the last 10 years, the surveillance and reporting of healthcare-associated infections (HAIs) or nosocomial infections has been done according to the last two Orders of the Ministry of Health, no. 916/ 2006 and no. 1101/2016. The 2006 order does not refer to the necropsy case report explicitly, but only to criteria that include a histopathological examination with a positive result for infection. Order no. 1101/2016 regarding the approval of the Norms for the supervision, prevention and limitation of infections associated with medical assistance in healthcare units uses the case definitions presented in Decision no. 2119/98/CE of the European Parliament and of the Council, from which we have chosen to present the subchapters in which a histological criterion similar to the definitions in order 916 of July 27, 2006 is indicated, as well as those in which necropsy criteria appear, respectively macroscopic aspects ascertained with the occasion of the autopsies, identifying only 3 situations (infection of the central nervous system, infection of the cardiovascular system, infection of the gastrointestinal system)[14].

Compared to the above, the contribution of the medical specialties that perform autopsies (forensic medicine, pathology) is reduced in terms of necropsy aspects related to case definitions, but it should be emphasized here that in the evaluation of a case the medical documentation is also available, respectively the results of laboratory analyzes and the clinical evolution during the hospitalization period.

Chapter 2. Microorganisms involved in AIAM

With regard to multi-resistant microorganisms specific to the hospital environment, the urgent threats indicated byThe Centers for Disease Control and Prevention and the World Health Organization are carbapenem-resistant Enterobacteriaceae, multidrug-resistant Acinetobacter, and multidrug-resistant Pseudomonas aeruginosa[15]occurring in healthcare-associated infections (HAI); the most challenging microorganisms developing resistance are summarized by the acronym ESKAPE: Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter spp.[16]. The National Institute of Public Health, Bucharest, Romania participates in the surveillance of antimicrobial resistance (AMR) in Europe, with national population coverage estimated at 21% in 2020, the

latest report showing an increase in carbapenemase-resistant strains of Klebsiella pneumoniae and Acinetobacter baumannii between 2016 and 2020[17].

Chapter 3. Postmortem bacteriological examinations

Healthcare-associated infections are a major concern for healthcare systems worldwide. The European Society for Clinical Microbiology and Infectious Diseases (ESCMID) Study Group on Forensic and Postmortem Microbiology (ESGFOR) evaluated postmortem microbiology sampling following in-hospital deaths.

The Study Group Consensus Statement identifies three main reasons to perform postmortem microbiology of biological samples: to identify the etiologic agent of a previously undiagnosed infection, to confirm a presumed antemortem diagnosis, and to determine the effectiveness of antimicrobial therapy[18]. Postmortem microbiological examinations (PMM) have been a controversial topic in the last century. Studies showing divergence from ante-mortem results[19] or a poor correlation between the type of infection and/or indicators of infection and the microorganisms recovered at autopsy[20] presents as the cause of events that occurred after death.

Agonal spread is the process by which bacterial invasion occurs during the death process or during resuscitation attempts due to ischemic damage induced on mucosal surfaces. Postmortem translocation is bacterial migration from the mucosal surface into the blood and internal organs after death[21]. Improper technical measures such as delayed cooling of the cadaver or sampling procedure, use of non-sterile instruments or operation in non-sterile environments can cause contamination[22].

In order to evaluate the value of postmortem microbiology cultures based on the existing literature, Riedel[2. 3] discuss the two main theories that explain bacterial growth in blood cultures and postmortem tissue cultures: agonal spread and postmortem bacterial transmigration, respectively. In addition, the author acknowledges the possibility of iatrogenic contamination of post-mortem samples during collection. Based on the literature reviewed, the relevance of post-mortem cultures for autopsies taken within the first 48 hours after death is supported, with a low probability of agonal spread and post-mortem bacterial translocation.

PERSONAL CONTRIBUTIONS

Working hypothesis and general objectives

Healthcare-associated infections raise difficulties in medico-legal practice regarding the role in the thanatogenic chain and in the context of allegations of deficiencies in the provision of medical-surgical assistance.

The general objectives of the doctoral study were:

- outlining the collection protocol in the study center

-identification of the chronology of the detection of antibiotic resistance types in postmortem microbiological examinations

- evaluating the subjective perception of medical examiners regarding healthcare-associated infections (HAIs)

- identifying the sensitivity of rapid tests for carbapenem resistance directly on biological products taken postmortem.

General research methodology

The evaluation of the general objectives of the doctoral research was carried out through 4 studies, as follows:

- Study I - Postmortem microbiology in forensic autopsies - a retrospective, monocentric study - Mina Minovici National Institute of Forensic Medicine, Bucharest, Romania (retrospective epidemiological study)
- Study II - Antimicrobial resistance trends - A monocentric retrospective study of healthcare-associated pathogens - patterns of post-mortem biological sample collection at forensic autopsies in Bucharest (retrospective epidemiological study)
- Study III- Questionnaire on healthcare-associated infections in medico-legal practice (prospective observational study using an online survey as a tool)
- Study IV - Rapid tests for carbapenem resistance performed directly on postmortem biologicals - a prospective pilot study (prospective experimental study)

6. Postmortem microbiology in forensic autopsies – a retrospective, monocentric study in Romania

6.1 Introduction

The Postmortem microbiology examination has been a controversial topic over the years, although the value of postmortem microbiology cultures remains promising. The aim of this

study was to review post-mortem microbiological sampling procedures and their results in a single center in Bucharest over a 10-year period.

Main objective: - outline the collection protocol in the study center

Secondary objectives: - assessment of postmortem contamination

- evaluation of postmortem bacterial translocation

- evaluation of the relevance of bacteriological examinations in medico-legal necropsy practice

6.2. Material and method

We performed a retrospective, observational, unicentric study at the Mina Minovici National Institute of Forensic Medicine in Bucharest, Romania, analyzing the results of post-mortem microbiological examinations and the corresponding autopsy files, related to the period 2011 to 2020. The collected data were included in an electronic database using Microsoft Excel 2010 software, statistical analysis was performed using IBM®SPSS Statistics for Windows version 24.0.

6.3. result

For the ten-year period (2011-2020) included in the study, we found a number of 630 cases in which microbiological investigations were requested. Microbiological analysis was requested in few cases compared to the total number of autopsies - between 1.89% and 5.59%.

The demographic characteristics of the study population were as follows: 245 women (38.9%) and 385 men (61.1%), age range 0 to 94 years, mean age 52 years. Deaths occurred in the hospital for 594 cases (94.3%) and outside the hospital for 36 cases (5.7% - field case).

For the analysis of sample type and microbiology we used all 630 cases, but for necropsy findings and medical records we could not retrieve 84 autopsy files, thus including in the statistical evaluation only 546 cases (522 hospital cases, 24 field cases). This was partly due to the fact that some autopsy files were requested and used in the processing of ongoing or pending civil or criminal cases for 2020 at the time of data collection.

Sampling patterns varied considerably: over the ten years we identified 104 different patterns for hospital cases and 19 patterns for field cases. For both subgroups, post-mortem blood cultures were the most common tests, followed by tracheal swabs/lung tissue samples and finally wound secretion swabs/skin tissue sample for hospital cases and harvest samples for field cases .

The sampling was performed in the first two days (48h) in 334 autopsies (53.0%) and in the first 4 days in 579 autopsies (91.9%). Exceptionally, for less than 1% of cases the autopsy was performed later than one week.

As a pathogen from the Enterobacteriaceae family, Klebsiella pneumoniae was the most frequently identified microorganism during the ten-year period. Acinetobacter baumannii and Pseudomonas aeruginosa were also among the top three pathogens. Staphylococcus aureus or other staphylococci were not organisms commonly identified in blood cultures in our study cases.

The number of microorganisms/culture did not vary significantly by case type, such that 70.8% of blood cultures for in-hospital death cases were polymicrobial, whereas 31.3% were monomicrobial for field cases ($p = 0.863$).

The comparison between the microorganism identified in blood cultures and the post-mortem interval identified only one statistically significant association, namely the result of Staphylococcus spp. (spp. – polymicrobial- family microorganisms) (median negative results 2.68 ± 1.28 days and positive results 3, 52 days ± 1.209, $p = 0.003$).

There was no statistically significant association between the post-mortem interval and the number of microorganisms identified ($p=0.122$).

Polymicrobial cultures were identified in 85.5% of cases with skin lesions reported at autopsy ($p=0.004$, $p<0.05$)). Acinetobacter baumannii and Pseudomonas aeruginosa (62.0%) were most frequently identified in the positive cultures, followed by Enterococcaceae (56.10%), regardless of the post-mortem interval.

We collected data on ante-mortem microbiology results from autopsy records for 233 (42.6%) of the in-hospital deaths of which 21 cases (9.1%) were negative. In this subgroup, there was an association between positive hospital microbiology samples and post-mortem microbiology samples (regardless of sample type) for Clostridium difficile ($p<0.001$), Klebsiella pneumoniae ($p=0.035$) and Proteus mirabilis ($p = 0.018$). In cases that had negative microbiology cultures in the hospital, only Staphilococcus aureus was identified in post-mortem cultures - 28.6% ($p=0.022$).

6.4. Discussions

Our data show that in-hospital deaths and field cases do not differ significantly in terms of the number of microorganisms identified in a positive blood culture. Although in postmortem

microbiology polymicrobial growth is considered contamination[24]–[26], there are also some studies reporting mixed-growth bacteria as genuine pathogens[27]. For our study population, the majority of swab samples from wound secretion or skin tissue were polymicrobial, regardless of the post-mortem interval, probably due to the circumstances of death (burn victims, trauma victims with prolonged hospitalization, immobilized patients with pressure ulcers known to have polymicrobial cultures)[28]–[30].

Although Clostridium spp., including Clostridium difficile[31], are fast-growing members of postmortem microbial cultures, respectively contaminants, our positive results for the latter were in faecal samples, with statistical correlation with infection documented before death.

Previous studies have suggested that postmortem spleen cultures may be useful in evaluating the effectiveness of treatment of antemortem bacteremia.[32], but spleen samples were rarely screened in our study cases, thus having insufficient data to evaluate the relevance of this type of sample. The PMM for in-hospital deaths can be used to assess response to treatment[18], however, rapid patient deterioration may have an unclear effect of antimicrobial treatment on postmortem culture results[33]. Our study did not aim to assess response to treatment, data extracted from medical records did not include clinical and laboratory onset of hospital infections or antibiotic treatment received, thus limiting the correlation with antemortem results. Postmortem assessment of treatment efficacy was a limitation of our study.

Collecting samples from at least two different sampling sites increased the likelihood of identifying an underlying (potentially fatal) infection, as did blood cultures and lung samples or from different collections.

7. Antimicrobial resistance trends – A monocentric retrospective study of healthcare-associated pathogens – post-mortem sampling at forensic autopsies in Bucharest

7.1.Introduction

Healthcare-associated infections are a major concern for health care systems worldwide. Microorganisms developing resistance to powerful antibiotics are an urgent threat to public health.

The present study aims to characterize antimicrobial resistance trends found in post-mortem microbiological samples taken during forensic autopsies in Bucharest, Romania, over a ten-year

period. Furthermore, we assessed the possibility that these examinations identify epidemiological outbreaks.

Main objective: - trends in antimicrobial resistance in postmortem bacteriological examinations at the study center

Secondary objectives: -evaluation of antibiotic resistance for the medical units included in the case report

-evaluation of antibiotic resistance of intrahospital bacteria

- evaluation of the possibility to identify reprospective outbreaks of HCAI

7.2 Materials and method

For the period 2010-2020, the autopsy files that met two inclusion criteria were selected, namely death occurring during hospitalization and the collection of biological samples for performing postmortem microbiology examinations. From the archive of the Forensic Laboratory of the National Institute of Forensic Medicine Mina Minovici, 516 forensic autopsy cases were selected that met the aforementioned criteria.

Cluster, AAMI outbreak was defined as multiple cases of AAMI occurring in a healthcare facility[34]in the same period of time. Because autopsies are not routinely performed on all in-hospital deaths and hospital autopsy rates continue to decline[35]we propose, for retrospective post-mortem epidemiology of AIAM, to use a calendar month as the unit of time. For the assessment, data on the medical facility is required. Regarding the duration of hospitalization, we stick with the definition of HCAI[36], infection identified more than 3 days after admission. Postmortem microbiology consists of positive results identifying the same pathogen with similar antimicrobial resistance (AMR).

7.3 Results

Analysis of microbiological samples was requested in several of the autopsies for in-hospital deaths performed in our center (1.68%-5.44% cases/year) and included in the study 1.62%-5.09% autopsies/year. We identified 516 forensic autopsies that met the inclusion criteria.

The demographic characteristics of the study population were as follows: 310 men (60.1%) and 206 women (39.9%), age range between 0 and 94 years, with a mean age of 57 years. The duration of hospitalization was between one (1) day and 232 days, with an average of 10 days; 401 patients (77.7%) were also admitted to intensive care units (ICUs) and had the longest hospital stays. The post-mortem interval, i.e. the period between death and the medico-

legal autopsy with the collection of biological samples was between one day (1) and 8 days post-mortem with a median of 2 days.

In order to evaluate the types of samples taken and outline a hypothetical collection protocol within the Mina Minovici National Institute of Forensic Medicine, we used the Missing Value Pattern function offered by IBM SPSS Statistics 24.0 for the 14 variables corresponding to the types of biological samples found to have been taken during retrospective analysis of autopsy records. Summary of missing values revealed that only 12.9% of the studied variables had complete data. Thus, 96 sets (combinations) of biological products were used for the 516 cases included in the study.

The general, increased antimicrobial resistance of enterobacteria is statistically significantly associated with the hospitalization period over 3 days ($p=0.018$, $p<0.05$).

Using the number of days of hospitalization as a continuous variable, a significant difference between the means of the groups is found, the number of days of hospitalization is higher for resistant enterobacteria ($F=6.538$, $p=0.011$, $p<0.05$) identified in positive postmortem blood cultures.

Regarding the periods of hospitalization in intensive care, although a directly proportional increase in resistant germs is observed in percentage terms, no statistically significant associations were obtained for the resistance types of enterobacteria.

It was found that with the increase in the hospitalization period, the number of resistant enterobacteria also increases for each postmortem blood culture, one resistant germ being identified at an average of 4.34 days and two germs at 4.82 days, respectivelyoutbreak of 3 multiresistant germs found at 8.2 days.

Pseudomonas aeruginosa and Acinetobacter baumannii were identified in blood cultures in less than 20% of cases. Multidrug-resistant Pseudomonas aeruginosa strains and carbapenemase-resistant variants were found in 5.5% of cases. P.aeruginosa was associated with prolonged hospitalization and ICU hospitalization. Resistant strains of P.aeruginosa appear to be more frequent with prolonged hospitalization (hospitalization < 3 days: 8.3% vs. hospitalization > 3 days: 32.8%) without reaching statistical significance ($p=0.087$). Positive blood cultures for Acinetobacter baumannii were associated with prolonged hospitalization ($p=0.046$,) while positive lung cultures for A. baumannii were associated with prolonged ICU stay ($p=0.015$).

The cases included in the study came from 29 medical units in Bucharest that were anonymized using Hn (H1-H29) and divided into 6 categories: 1-Emergency Hospital, 2-Chronic Hospital, 3-Children's Hospital, 4 -Hospital of Infectious Diseases. , 5-Private Hospital, 6-Maternity. The cases included in the study mostly came from emergency hospitals (69.8%), followed by chronic disease hospitals (12%). Antibiotic-resistant strains (ESBL, CP, MDR - predominantly A. baumannii) were mainly identified in emergency hospitals, more than 75% of resistant strains, and vancomycin-resistant enterococci were found only in this type of unit.

Post-mortem microbiology shows a retrospective picture of AMR in hospitals in Bucharest, Romania, between 2011 and 2020: ESBL variants are predominant with a significant peak in 2016, CR variants (carbapenemase-producing and non-carbapenemase-producing), were rare (< 10 cases). /year) until 2019 when they surpassed ESBL variants. Other MDR and VR isolates have a low but consistent identification rate of less than 10 cases/year.

Thus, for the study period, we recovered a maximum of 6 cases from the same medical unit that were medico-legally autopsied in the same month. We attempted a retrospective analysis of the main virulent microorganisms in the hospital environment, namely Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp. known under the acronym ESKAPE.

Thus, 11 clusters with the aforementioned microorganisms were recovered, the deaths occurred in only 3 medical units (H1 and H2 are Emergency Hospitals in Bucharest, H17 - Hospital for Chronic Diseases in Bucharest).

7.4 Discussions

Antimicrobial-resistant pathogens in hospitalized patients are of great concern to physicians worldwide.

Postmortem microbiology has had questionable relevance in the past, but has gained relevance in sampling from hospital deaths through systematic research.

This is the first systematic single-center study that describes the identification of AMR variants of microorganisms specific to hospital environments recovered from post-mortem sampling during forensic autopsies in Romania.

However, this retrospective analysis has several limitations that contribute to an underestimation at the clinical level.

First of all, the number of medico-legal autopsies in which post-mortem microbiology was requested is small, because this type of investigation is not a standard in autopsy protocols in Romania and is requested on a case-by-case basis by the medical examiner.

Second, the heterogeneous sampling patterns identified in our study consisted of 14 sample types in 96 combinations that interfere with the association of microbiological results and histologically confirmed infection/site of infection. This variation necessitates a standardized sampling protocol.

Thirdly, according to Romanian legislation, not all hospital deaths are subject to a medico-legal autopsy.

Therefore, these post-mortem results are applicable to a small proportion of cases: violent or suspicious deaths for which a medico-legal autopsy is ordered by the authorities, which causes an underestimation of AMR by the present study; epidemiological indicators such as incidence and prevalence are considered irrelevant to our study.

Other limitations include the unavailability of data on medical history and previous hospitalizations that would serve to outline a more complex epidemiological picture.

8. Questionnaire on healthcare-associated infections in forensic practice

8.1.Introduction

In medical practice, the use of surveys on target populations (patients, medical personnel) can be a tool for improving the quality of services[37],[38].

Survey research is a common method for medical or health service studies. The questionnaires used are structured to obtain a picture at a certain point in time, being predominantly suitable for descriptive studies.

The aim of this study was to evaluate the opinion of medical-forensic professionals on the necropsy and clinical-forensic casuistry regarding healthcare-associated infections (HAI).

Main objective - eassessing the subjective perception of medical examiners regarding healthcare-associated infections (HAI) in practice

Secondary objectives - assessment of judgment regarding necropsy practice in cases with AIAM

- assessment of judgment regarding clinical medico-legal practice in cases with AIAM

8.2.Material and method

An observational study was conducted using an online survey entitled "Questionnaire on healthcare-associated infections in forensic practice" as an instrument. Collected data were entered into an electronic database and statistical analysis was performed using IBM®SPSS Statistics for Windows version 24.0.

8.3.result

24 responses were collected.

We present the results structured in 3 categories: socio-professional data; issues related to casuistry; aspects of professional judgment.

Related to casuistry, it seems that respondents rarely encountered objective medico-legal works related to HCAI (17 respondents, 70.8%), more frequently in works of the new expertise type (10 respondents, 41.7%), predominantly in cases of traumatology in people who also had pre-existing pathological conditions (17 respondents, 70.8%), with death following the infection (20 respondents, 83.3%). The cases predominantly came from surgery wards.

Specialist opinions were rarely requested (infectious diseases/epidemiology), when drawing up the conclusions, the elements provided for by national legislation, epidemiological investigations, antibiotic resistance of identified microorganisms and specialist opinions were predominantly taken into account;

With regard to professional judgment, we used questions with scalable answers and we emphasize that the respondents' approach was applied to the case, the simple identification of an AIAM in a case does not guide the presence or absence of deficiencies in the provision of medical assistance.

- the majority of respondents do not consider HCAI a deficiency if it was identified and treated, but in situations where it was identified and not treated or not identified and implicitly not treated;
- in cases of traumatology, the respondents considered that the appearance of an IAMI can at most influence the type of causal link, without being obligatory - always - an indirect causality

8.4.Discussions

Among the limitations of this research we have the small number of respondents that provide a partial picture and the fact that the survey was only at the national level;

9. Performance evaluation of RESIST-5 OKNVI assays for postmortem biological samples - Prospective pilot study

9.1. Introduction

In this study, the performance of RESIST-5 OKNVI assays was evaluated as the ability to detect and differentiate the five isoenzymes (carbapenemases) in impure postmortem biological samples and pure bacteriological cultures resulting from these samples as a reference standard.

The purpose of the studyis to determine the sensitivity and specificity of rapid tests for postmortem carbapenem resistance in biologicals, as well as the feasibility of rapid tests and to see if they can assist the medical examiner/pathologist regarding the appropriateness of postmortem carbapenem resistance testing.

Main objective -Identification of the sensitivity of rapid tests for carbapenem resistance directly on postmortem biological products.

Secondary objective -Semiquantitative comparison of the presence of bacteria in biological samples

9.2. Material and method

We conducted a prospective, single-center pilot study evaluating on-site carbapenemase resistance testing from post-mortem specimens versus testing from pure cultures after inoculation, isolation, and identification of causative agents.

The inclusion criteria of the patients were as follows: length of hospitalization greater than 3 days, a carbapenem-resistant (CR) bacterium identified before death, post-mortem bacteriology ordered by the pathologist to evaluate the cause of death.

During the autopsy, four swabs were collected for each specimen as follows:

•Tampon I was tested on site, immediately after sampling. The buffer was dipped into a semi-rigid tube containing 12 drops of LY-A buffer, mixed well, and the diluted sample was pipetted onto each of the two RESIST-5 OKNVI cassettes, the results were read after 15 minutes (test I).

•Tampon II was tested in the local laboratory. The swab was used to inoculate BrillianceTM ESBL Agar (Oxoid, Basingstoke, UK) - a chromogenic screening plate for the detection of extended-spectrum ß-lactamase-producing organisms. BrillianceTM ESBL agar plates were examined after incubation at 36ºC ± 2ºC for 18 to 24 hours. If the agar showed mixed populations, an isolation plate was obtained to further separate individual microorganisms.

Once pure cultures were obtained, the RESIST-5 OKNVI assay was performed according to the manufacturer's instructions (assay II). Results were obtained in 1-2 days, depending on culture growth and incubation facilities.

9.3. result

A total of 200 autopsies were performed at the Mina Minovici National Institute of Forensic Medicine in the months of June - July 2022. We identified 21 forensic autopsies that met the inclusion criteria and for 14 of them complete data were available and had were included in the present study.

We performed 39 RESIST-5 OKNVI rapid tests on 19 biological samples, at least one sample/case, for 5 cases two biological samples were collected. For two samples the first tests were negative and after 24 hours of incubation no microorganisms grew on Oxoid Brilliance ESBL Agar, so for these samples the second set of tests was not performed.

In the study samples, the RESIST-5 OKNVI tests directly from biological samples showed an overall sensitivity of 92.3% and a specificity of 100%, positive predictive value 100%, negative predictive value 80.0%. In the second set of tests for 10 cultures, the results were positive for 2 types of CR isozymes.

No IMP variants were identified in our study, VIM variants were present in 2 cases, NDM variant in 10 cases, KPC in 3 cases and OXA-48 in 8 cases.

For the KPC variants we recovered a sensitivity of 100% with a specificity of 92.9%. For VIM and NDM the overall specificity was 100%, but the sensitivity was below 80%. OXA-48 variants were identified directly from biological samples with a sensitivity of 75.0% and a specificity of 77.8%.

The post-mortem interval for sampling did not show a statistically significant influence on test II results (p=0.212) when we compared samples taken in the first 24 hours with those taken later up to 6 days, and the concordance of test I and test II for the CP variants did not demonstrate. change with postmortem interval in days (NDM [F(5,13)=0.99, p=0.460], VIM [F(5,13)=1.09, p=0.409], KPC [F(5,13)=0.35, p=0.870], OXA-48 [F(5,13)=2.58, p=0.078]).

9.4. Discussions

Carbapenemase-producing isolates are increasing both in the hospital and in the community[39]**,**as well as in animals[40]. For the practice of forensic medicine, these infections raise several issues with respect to negligence allegations that focus on undiagnosed infections associated with healthcare or treatment/antibiotic before death.

Since postmortem bacteriology is rarely requested during forensic autopsies at our center, in part because they are expensive and time-consuming examinations, and because rapid tests with postmortem applications are under development, we wanted to see their performance and in post-mortem sampling.

The overall sensitivity and specificity of the RESIST-5 OKNVI for postmortem sampling was satisfactory. We believe that these rapid test results, directly from the biological specimen and not from the culture, were due to the quality of the specimen, undiluted as tracheal/bronchial lavage

Post-mortem sampling for bacteriology is recommended to be performed within the first 24 hours[18] after death, however, the post-mortem collection interval for our samples did not affect the concordance of results with assays performed directly from bacterial cultures.

Although limited by the small sample size, our results showed a good correlation between the assay performed directly from biological samples and the result from pure cultures after 24/48 hours of incubation.

10. General Discussions

The lack of a PMM sampling protocol is also illustrated by the heterogeneity of patterns identified in our data over a 10-year period (104 different patterns for hospital death cases and 19 patterns for field cases)[41].

Similar to previous studies in the field, our data support contamination of the sample, when the result of the postmortem microbiological examination is Staphylococcus spp. ($p=0.003$), respectively a polymicrobial blood culture from this family[26], [42], [43]. Recent studies in animal models show that from day 2 to day 18 postmortem Staphylococcus spp. are the predominant genus identified as a harvest contaminant[44].

Carbapenemase-producing Enterobacteriaceae (CPE) isolates from our post-mortem microbiology results increased in frequency in 2019[45], an aspect that also coincides with the reports of public health authorities in Romania[17]. ESBL variants decreased as CP increased, possibly due to an increase in the use of carbapenem therapy. AMR variants of major concern nowadays, such as vancomycin-resistant S. aureus or CP A. baumannii were not identified in our

samples. Vancomycin-resistant enterococci were not isolated in the first half of the study period (2011-2014).

Our attempt to describe AIAM outbreaks by post-mortem microbiological results and retrieval of epidemiologic clusters/outbreaks had several limitations, mainly due to the small number of cases/medical unit/month.

. The inclusion of the legal medicine specialty in Romania in a system of supervision of HCAI at the national level would require, for feasibility, a phasing.

Regarding the questionnaire completed online by specialists in the field, it is interesting that we found a high number of respondents who consider it appropriate to involve legal medicine in supervision, even in the absence of an explicit legal framework.

In the 4th study, the rapid tests used were complex and tested 5 carbapenemases variants, but as we recovered no IMP variants and only two VIM variants in 19 samples, we suggest that earlier versions would be sufficient, such as RESIST-4 OKNV[46][47] or RESIST -3 OKN[48], for post-mortem screening of CP-CR microorganisms[49]. In gross findings of infection with or without a previously known etiologic agent, screening for carbapenem resistance may guide the ordering of a complete postmortem bacteriological examination.

Conclusions

Based on the results of the four studies carried out as part of the doctoral research, we formulate the following conclusions:

Regarding the general objective of the first study, it was found that the postmortem collection of biological samples for microbiological examinations in the study center is heterogeneous, with a wide variety of patterns (104 patterns for death cases in medical facilities and 19 patterns for field cases).

At the same time, a correlation was established between the results of post-mortem cultures, taken from different anatomical sites, with macroscopic necropsy aspects and histological findings, which can allow the coroner to identify the etiological agent of the antemortem infection and provide significant help in establishing the exact cause of death .

Our results were promising in reconfirming a microorganism identified antemortem in medical facilities.

A positive blood culture result for Staphylococcus spp., without identification of a specific microorganism, is more likely due to postmortem contamination than its role as an etiological agent.

In the second study, on antimicrobial resistance trends, the results showed that carbapenemase-producing variants of Enterobacteriaceae species were identified as early as 2011 and remained relatively constant until 2019, when a significant increase was noted which also coincided with the data reported by public health aothorities at the national level, being at that time an element of concern for medical institutions. Resistance to vancomycin in the study group was identified only in Enterococcus species (E. faecium and E. faecalis) as of 2015.

Secondarily, the presence of pathogens known by the acronym ESKAPE and their antimicrobial resistance were associated with prolonged total hospital stay, respectively hospitalization in intensive care units (more than 3 days).

Our results were promising for the use of post-mortem microbiology in forensic practice as a method to identify healthcare-associated infections (HAI) in high-risk facilities and may be a useful additional method for HAI surveillance. The proposed criteria for identifying postmortem epidemiologic clusters/outcries were able to identify 7 possible HCAI clusters by retrospective analysis, a promising result for further prospective studies.

Regarding the issue of healthcare-associated infections in forensic practice, in the third study, the responses of forensic professionals showed that in practice there is an approach applied to the case, an infection associated with healthcare was considered by the majority respondents deficiency in the provision of medical/medical-surgical assistance in two situations: if the infection was not identified and implicitly not specifically treated or if the infection was confirmed but not treated.

The results of the fourth study showed that the use of RESIST-5 OKNVI tests on biological samples collected from the cadaver during the medico-legal autopsy, although on a small study lot, proved useful and the results were observed to be feasible, respectively identification of the presence and type of carbapenemase, directly from the collected sample, without the need for cultures, withan overall sensitivity of 92.3% and a specificity of 100%,representing an element of predictability with increased specificity for the post-mortem diagnosis of these infections.

It has also been observed that the use of the post-mortem screening procedure for carbapenem resistance helps to select cases for further analysis with complete post-mortem bacteriological examinations.

Personal contributionof the research in the doctoral thesis consists of:

1. Demonstration of the role of post-mortem bacteriological examinations in the identification of the etiological agent involved in the thanatogenerative chain, the results of the study showing a correct association between positive cultures and the site of infection confirmed macro or microscopically.

2.Exemplifying the role of forensic medicine in the issue of antimicrobial resistance surveillance, showing that retrospective post-mortem studies tend to provide a mirror image of the situation in medical facilities.

3.The proposal of some postmortem criteria for the identification of outbreaks of infections associated with healthcare, coming in support of national public health policies, with promising results, which lend themselves to prospective studies, possibly as a constitutive element of some national surveillance systems.

4.Evaluation of the sensitivity and specificity of rapid tests for the identification of carbapenem resistance in a prospective study, being the first research to directly address their applicability to necropsy samples with autopsy testing.

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1. **Devil I**, Keresztesi AA, Cerghizan AM, Negrea M, Dogăroiu C. Postmortem Bacteriology in Forensic Autopsies—A Single Center Retrospective Study in Romania. Diagnostics. 2022; 12(8):2024.

   Access link:https://doi.org/10.3390/diagnostics12082024
   Magazine Indexing –PubMed, Impact Factor – 3.6
   Chapter 6 – Personal Contribution (pp. 43-71)

2. **Devil I.**, Dogăroiu C, Keresztesi AA, Horumba M. Antimicrobial resistance trends - a single-center retrospective study of healthcare-associated pathogens - postmortem sampling from medico-legal autopsies in Bucharest. Germs. 2022 Sep 30;12(3):352-360. PMID: 37680676; PMCID: PMC10482475

Access link:https://doi.10.18683/germs.2022.1339
Magazine Indexing –PubMed Central, Impact Factor – N/A
Chapter 7 – Personal Contribution (pp. 72-108)

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   Magazine Indexing –PubMed, Impact Factor – 2.7
   Chapter 9 – Personal Contribution (pp. 122-134)