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PERFORMANCE OF THE RIBEIRÃO PRETO REGIONAL LABORATORY CENTER - VI OF THE ADOLFO LUTZ INSTITUTE IN THE RESEARCH OF EPIDEMIOLOGICALLY RELEVANT ETIOLOGICAL AGENTS

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Abstract: Introduction: the Ribeirão Preto Regional Laboratory Center - Instituto Adolfo Lutz (CLR-RP-IAL-VI) serves the Regional Health Care Network 13 (RRAS-13), as a Reference Laboratory (LR) for cases of Bacterial Meningitis (MB) and Health Care Related Infections (IRAS), acting in the characterization of bacteria and fungi recovered in cultures carried out in Local Laboratories (LLs). **Goal**: to present the results obtained with the isolates of interest in public health sent by the LLs sent to the LR for characterization studies, phenotypic identification, bio/serotyping and research of antimicrobial resistance. **Methodology:** retrospective study describing investigative results of bacterial and yeast isolates sent to CLR-IAL-RP-VI by LLs belonging to RRAS-13, from January 2015 to November 2020. **Results:** A total of 843 isolates of bacteria and 17 of yeast fungi were obtained from cultures carried out in the LLs for the diagnosis of Meningitis (146 bacteria and 17 yeasts), Pneumonia (126 per *Streptococcus pneumoniae* and 23 other bacteria), IRAS (353 Infections by Multi-Drug Resistant Bacteria - InfecBMR; 73 sepsis and 108 other diseases). Among 146 MB isolates, 86 were of epidemiological importance(5 *Haemophilus influenzae*; 22 *Neisseria meningitidis* and 59 *S. pneumoniae*). Of the 353 InfecBMR isolates identified: 202 *Klebsiella pneumoniae* (109 ESBL producers, 159 Carbapenemase producers and 81 carriers of both genes); 14 *Enterobacter cloacae* (10 resistance gene carriers = 5 *amp*C; 1 *ESBL*; 3 KPC e 1 NDM); 23 *Escherichia coli* (11 showed resistance genes = 4 *ESBL*; 4 KPC; 1 *ESBL*+KPC; 1 *mcr-1*e 1 *bla* OXA48) e 1 *Klebsiella* sp NDM. In cases of fungal meningitis, 14 were *Cryptococcus neoformans* and 3 *Cryptococcus gattii*. **Conclusion:** The diversity of characterized etiological agents values the importance of LR in complementary studies that are of great

importance for epidemiological surveillance actions.

Keywords: Meningitis, Pneumonia, Fungi, Carbapenemases, IRAS.

INTRODUCTION

Instituto Adolfo Lutz (IAL) is a Public Health Laboratory in the State of São Paulo and is directly linked to epidemiological and sanitary surveillance services. Linked to the São Paulo State Health Department and integrated into the Disease Control Coordination, the IAL consists of a Central Laboratory with 10 Technical Centers and 12 Regional Laboratory Centers (CLR-IAL). The work of IAL is mainly in the areas of Medical Biology, Pathology, Bromatology and Chemistry. Considered the largest Public Health Laboratory in Latin America, it performs specialized and differentiated activities, generating information relevant to public health and scientific knowledge. Due to the activities being carried out in a network system, it is up to the Central IAL, in addition to carrying out more complex tests, the supervision of other laboratories, the development and standardization of laboratory techniques and methods (SAÚDE, 2022; TRUJILLO et al., 2012).

The Ribeirão Preto Regional Laboratory Center - Instituto Adolfo Lutz - VI (CLR-RP-IAL-VI) is a Reference Laboratory (LR) for the control of notifiable diseases and health problems, occurring in Municipalities covered by the Regional Network of Health Care 13 (RRAS-13), which is composed of four Regional Health Departments (DRS): i- Barretos located in the Northwest macro-region of the state of São Paulo; ii- Araraquara; iii- Franca and iv- Ribeirão Preto, which are located in the Northeast macro-region of the state of São Paulo, totaling 90 municipalities (RRAS 13, 2014).

Patients treated in health services covered by RRAS-13 have their clinical samples sent to Local Laboratories (LLs) for microbiological diagnoses. In cases of notifiable diseases including meningitis, pneumonia and Health Care-Related Infections (HAI), especially in the investigation of Infections caused by Multidrug-resistant Bacteria (InfecBMR), etiological agents of epidemiological interest must be referred to the CLR-IAL-RP-VI for additional epidemiological characterization studies including phenotyping, bio/serotyping and antimicrobial resistance research involving polymerase chain reaction (PCR) assays, while other more complex studies are carried out at IAL Central. Based on the results obtained and published, the Epidemiological Surveillance Services determine actions such as: chemoprophylaxis, vaccination, isolation measures and prevention of the spread of multidrug-resistant agents.

GOAL

To present the results obtained with the isolates of interest in public health sent by the LLs sent to the LR for characterization studies, phenotypic identification, bio/serotyping and research of antimicrobial resistance.

METHODOLOGY

This is a retrospective study describing investigative results of bacterial and fungal isolates forwarded to CLR-IAL-RP-VI by LLs belonging to RRAS-13, from January 2015 to November 2020. A total of 860 strains of microorganisms, being 843 bacteria and 17 yeasts recovered from cultures performed in the diagnoses of: 146 cases of bacterial meningitis (MB); 17 cases of fungal meningitis (MF); 149 cases of pneumonia; 548 cases of HAI and 108 cases of other diseases.

Cultures of the following clinical samples were performed: 9 Sputum; 8 Feces; 3 Fragments of skin; 2 Bone fragments; 1 gastric lavage; 4 Bronchoalveolar lavages;

7 Abdominal fluids; 26 Pleural fluids; 105 Cerebrospinal Fluids; 26 Catheter tips; 1 Corneal scraping; 334 Blood cultures; 1 Vaginal secretion; 1 Oropharyngeal secretion; 3 Bronchial secretions; 12 Wound secretions; 6 Nasal secretions; 11 Eye secretions; 83 Tracheal secretions; 1 Urethral secretion; 66 Anal Swabs and 146 Urines.

Bacterial isolates were subjected to phenotypic characterization and identification, following traditional methodologies and determined by clinical microbiology manuals. They were initially characterized according to the morphology observed in Gram stain, followed by growth in different culture media, biochemical and serological tests (HOLT et al., 1994; JORGENSEN et al., 1999; BALOWS et al., 1991; WAUTERS & VANEECHOUTTE, 2015; BRAZIL, 2013).

The Antimicrobial Sensitivity Tests performed followed criteria established by the CLSI (Clinical & Laboratory Standards Institute), performing the Disc Diffusion (DD) and Minimum Inhibitory Concentration (MIC) methods using E~test (CLSI, 2015, 2017, 2018, 2019, 2020; BRAZIL, 2013).

The DD method is qualitative and determines the sensitivity to antimicrobials, allowing the presumptive detection of resistance mechanisms, especially *ESBL* (Extended-Spectrum Beta-Iactamase) and *ampC*, that indicate a pattern of resistance to beta-lactam antimicrobials. All isolates suspected of InfecBMR were investigated to determine resistance phenotypes with enzyme blockers (RIBEIRO, 2016). The presence of carbapenem resistance genes (KPC) and *New Delhi metallo-beta-lactamase* (NDM) was confirmed by performing real-time PCR (BRASIL, 2020).

Yeast fungus isolates were subjected to phenotypic characterization and identification, following traditional and established methodologies for the identification of *Cryptococcus* sp (MARTINS et al., 2017; KWON-CHUNG et al., 1982; CUENCA-ESTRELLA et al., 2002; CLAUDINO et al., 2008).

RESULTS AND DISCUSSION

Table 1 presents the distribution of the 843 cases of bacterial diseases investigated, highlighting the numbers of meningitis, pneumonia and BMR infec, with the latter standing out in numbers among the other diseases, coinciding with the increase in hospitalization and invasive procedures related to COVID19.

Regarding Bacterial Meningitis (MB), the occurrence of any species involved must be valued, however, from an epidemiological point of view, the etiological agents of greatest importance in public health are *Haemophilus* sp, *Neisseria meningitidis* and *Streptococcus pneumoniae*, which have several serotypes that, when prevalent, can define vaccination schedules as well as patient treatment and chemoprophylaxis of contacts (SALGADO et al., 2012).

Among the 146 cases of bacterial meningitis investigated, 86 isolates of epidemiological importance were recovered, as follows: 5 *Haemophilus influenzae*; 22 *Neisseria meningitidis* and 59 *Streptococcus pneumoniae*. The rest of the isolates were identified as Gram Negative Bacteria: (2 *Acinetobacter* sp; 2 *Aeromonas caviae*; 1 *Alcaligenes faecalis*; 2 Asaccharolytic non-fermenting gram negative bacilli; 2 *Burkholderia* sp; 4 *Escherichia coli*; 1 *Klebsiella pneumoniae*; 1 *Neisseria gonorrhoeae*; 1 *Pseudomonas fluorescens*; 1 *Stenotrophomonas maltophilia*) e Bactérias Gram Positivas: (4 *Bacillus* sp; 4 Gram Positive Coryneform Bacillus; 1 *Listeria monocytogenes*; 1 *Micrococcus* sp; 2 *Nocardia* sp; 1 *Rhodococcus* sp; 1 *Sarcina* sp; 13 *Staphylococcus* sp Plasma Coagulase Negative;1 *Staphylococcus* hyicus;

INVESTIGATED CLINICAL SUSPECTS						
YEAR	**Nº**	**Meningitis**	**Pneumonia**	**InfecBMR**	**Sepsis**	**Others**
2015	187	30	30	72	24	30
2016	165	25	19	58	33	30
2017	90	26	30	13	03	18
2018	106	30	33	14	14	15
2019	198	24	26	132	09	07
2020	97	11	11	64	04	08
TOTAL	843	146	149	353	87	108

Observations: those cases of infections whose clinical features differed from meningitis and pneumonia were considered as sepsis, and the diagnosis was concluded through a positive blood culture. Less frequent health problems were included as other suspicions, including (Conjunctivitis; Convulsive crisis; Fever to be clarified; Typhoid fever; Acute infectious gastroenteritis; Abdominal infections; Skin infections; Genital infections; Liver infections; Eye infections; Lung infections ; Respiratory infections; Pyelonephritis and Polyarthritis).

Table 1. Distribution of 843 cases of investigated bacterial diseases, whose isolates were sent to CLR-RP-IAL-VI for further identification, from January 2015 to November 2020.

Nº	**Bacterial isolate**	**Resistance Mechanisms**			
		ESBL	**KPC**	**Both**	**Others**
01	Citrobacter freundi	00	01	00	00
06	Enterobacter aerogenes	03	02	01	00
14	Enterobacter cloacae	01	03	00	AmpC[5]; NDM[1]
23	Escherichia coli	04	04	01	mcr-1[1]; bla oxa48[1]
01	Klebsiella oxytoca	01	01	01	00
202	Klebsiella pneumoniae	109	159	81	00
11	Klebsiella sp	05	05	03	NDM[1]
03	Raoutella terrigena	02	02	01	00
Total = 261 isolates		**125**	**177**	**88**	**09**

Abbreviations: ESBL= Extended Spectrum Beta Lactamase, KPC= Carbapenemase-producing Klebsiella pneumoniae. mcr-1= Mobilized Colistin Resistance. AmpC= cephalosporinase that hydrolyzes penicillins, including traditional β-lactamase inhibitors (Clavulanate, Sulbactam and Tazobactam), and cephalosporins up to the third generation. NDM= New Delhi Metallo-betalactamase.

Table 2. Occurrence of 261 Isolates of Gram-Negative Fermenting and Multidrug-resistant Bacillus, with appropriate Resistance Genes, during the period from 2015 to 2020.

1 *Staphylococcus aureus*; 4 *Streptococcus* sp; 1 *Streptococcus pyogenes*) and 9 isolates were indeterminate).

Community-acquired pneumonia is considered one of the leading causes of death worldwide. Although mortality rates from respiratory infections have been significantly reduced in recent decades, this condition continues to rank third in our country. The respiratory microbiota consists of a vast microbial population, where *Streptococcus pneumoniae* remains the most prevalent bacterium among the etiological agents involved in these infections (CORRÊA et al., 2018). Of the 149 cases of pneumonia investigated, *Streptococcus pneumoniae* was identified in 126 and other bacterial agents were identified in the remaining 23.

In the last decades, nosocomial infections have been fought systematically, mainly in developed countries. As of 1990, the term "Hospital Infections" was replaced by "Health Care Related Infections" (IRAS). This designation is considered a conceptual expansion, incorporating infections acquired and related to care in any environment. In fact, HAIs represent a great impact on hospital patient lethality, when we considered the length of hospital stay, increased severity of the disease, in addition to the induction of antimicrobial resistance. Therefore, all this gives HAI special relevance to public health (PADOVEZE & FORTALEZA, 2014). HAIs consist of adverse events that are still persistent in health services. It is known that infections considerably increase the costs of patient care, in addition to increasing hospital stay, morbidity and mortality (BRASIL, 2016). The following situations are determined as HAIs: surgical site infection, primary bloodstream infection, respiratory tract infection, urinary tract infection and other infections covering the following sites: ears, nose, throat, mouth, cardiovascular system, skin and tissues moles, gastrointestinal tract and reproductive tract (BRASIL, 2017a, 2017b).

Considered as the most relevant HAIs, BMR infections represent a growing threat to public health, being normally found in hospital environments due to the selective pressure of antibiotics used on a large scale, and may also occur in an extra-hospital way. The occurrence of BMRinfect requires special attention with regard to its diagnosis, transmission control and appropriate treatment. The Epidemiological Surveillance Center (CVE) is responsible for the surveillance and monitoring of the incidence of BMR infection in the State of São Paulo. Based on the result of a multidrug-resistant bacterial isolate, it is essential that the LL send the sample to the LR for confirmation and determination of resistance genes through molecular biology. This same procedure must be followed in cases of InfecBMR outbreaks, however, accompanied by notification to the CVE (SES-SP, 2016; CHAGAS et al., 2016).

Among 353 cases of InfecBMR investigated, 289 isolates of Gram Negative Bacillus fermenting glucose were shown to be multidrug resistant, among which 261 showed at least one resistance mechanism. Table 2 shows the higher occurrence of Klebsiella pneumoniae producing Extended Spectrum Beta-Lactamase and Carbapenemases. Among 14 Enterobacter cloacae isolates, 10 of them carrying resistance genes (5 ampC; 1 ESBL; 3 ESBL and KPC; 1 NDM) were also determined. Among 23 Escherichia coli isolates, 9 showed resistance genes (4 ESBL; 4 KPC; 1 mcr-1 and 1 bla OXA48), and 1 Klebsiella sp isolate carrying the NDM gene was also identified.

The event of fungal infection, especially meningitis, health care institutions must be vigilant, due to its progressive increase and high rates of morbidity and mortality. In general, the individuals most prone to infections are those who are immunocompromised, and they may be infected endogenously and/or exogenously, with the latter situation coming from various sources of contamination. Among the most prevalent opportunistic fungal infections are candidiasis, aspergillosis, mucormycosis and cryptococcosis (NAKAMURA et al., 2013). Infections caused by Cryptococcus sp are of great epidemiological importance because they cause, in addition to skin diseases, pneumonia and meningitis in immunocompromised or immunocompetent patients. This study revealed 17 cases of fungal meningitis, with 14 isolates of Cryptococcus neoformans and 3 of C. gattii being recovered in culture. These species are the most incident and despite promoting disease in humans, both denote different clinical and epidemiological aspects, being C. gattii responsible for infections in immunocompetent patients and may be more refractory to treatment with fluconazole (MINISTRY OF HEALTH, 2012).

CONCLUSION

The diversity of etiological agents of epidemiological interest, referred to the CLR-RP-IAL-VI for further studies underscores the importance of LLs being at the forefront of their primary isolation, their notification and submission to the LR. This study values the importance of the LR in terms of fulfilling its mission vis-à-vis public health services as a provider of specific information that are considered tools for epidemiological surveillance actions to be carried out in the fight and control of diseases and/or health problems.

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