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Identification of *Salmonella enterica* serovar Typhi strain from a young Brazilian patient: the relevance of automated microbiological methods for the rapid diagnosis of systemic infections

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Abstract

Introduction: Latin American countries have public health problems that are aggravated by inadequate basic sanitary conditions. Inefficient vaccination coverage, as well as the fact that a significant portion of the population is exposed to contaminated water and food, can lead to an increase in the incidence of some diseases. Methodology: the work reports a case of infection by *Salmonella enterica* serovar Typhi (S. Typhi), whose contagion is strictly related to personal and environmental hygiene conditions. Conclusion: accurate identification and immediate detection of the etiologic agent using automated methods, such as Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) and the BACT / ALERT System® 3D, can positively contribute to the patient's prognosis and therapeutic success. Thus, principally in the Latin American, a quick and adequate treatment is of great importance, both for the patient and to minimize the spread of multidrug-resistant strains and the consequent reduction of therapeutic options.

Key word: Salmonellosis, *Salmonella* Typhi, Typhoid fever, MALDI-MS, Blood culture.

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Introduction

Typhoid fever is an acute systemic infectious disease that has millions of cases worldwide and is responsible for thousands of deaths, remaining a major public health problem. It is caused by *Salmonella enterica* serovar Typhi (S. Typhi), a Gram-negative, aerobic, and non-spore-producing microorganism of the Enterobacteriaceae family. This infection can lead to symptoms ranging from nausea, prolonged fever, diarrhea and abdominal pain to severe systemic conditions such as septic shock, acute renal failure, liver dysfunction and neurological complications (1-6).

As a public health problem, typhoid fever is associated with poor sanitary conditions and is present in developing countries, where an important amount of the population is exposed to contaminated water and food. Although there is a vaccine to prevent the disease, in Brazil, the vaccination is only indicated in specific situations, such as for recruits on mission in endemic countries, workers who come into contact with sewers or people living in highly endemic areas (7,8).

A rapid laboratory diagnosis has a great impact on the treatment of systemic infections, reducing mortality rates, hospitalization and costs. However, factors such as the duration of fever and previous use of antibiotics, together with the limited availability of services, influence the diagnosis, which can lead to underreporting of cases (9-11). Until recently, microbial identification occurred mainly by conventional phenotypic techniques or by gene sequencing, undergoing a revolution with the use of matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS) which became decisive in the identification of unusual microorganisms in laboratory routine. It is also assumed a better assertiveness in identifying species that can be neglected using conventional techniques (12).

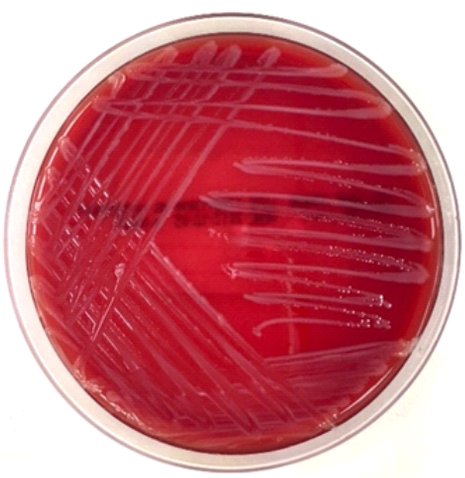
Thus, given the possibility of non-detection, misidentification and clinical worsening, the present study aims to report a systemic infection by S. Typhi whose diagnosis was aided by the use of automated microbiological methods.

Case report

A 20-year-old male patient was seen by his general

practitioner in the city of Macapá, Amapá, Brazil. On clinical examination, he complained of tiredness, fatigue, constant fever, and abdominal pain that persisted for 10 days. Two blood samples were collected and incubated in the BACT/ALERT® 3D system (bioMérieux®, Brazil). After approximately 4 hours of incubation, the automated system indicated positivity in both blood culture vials. An aliquot was sowed in blood agar plate (bioMérieux®) and incubated at 37 °C for 24h. After the incubation period, it was observed the growth of non-pigmented and mucoid colonies (Figure 1).

Figure 1: Microbiological features of Salmonella enterica serovar Typhi strain isolated from blood samples of a young Brazilian patient. Colonial morphology on 5% sheep blood agar plate

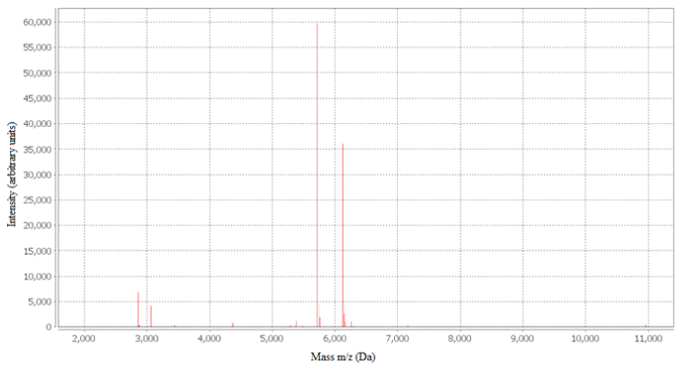


The identification of the microorganism was performed by MALDI-TOF MS (VITEK® MS, bioMérieux®), preparing a protein extract of isolates grown in blood agar at 37 °C for 24 h suspended in 10% formic acid (bioMérieux®) which identified the sample as S. Typhi (99% probability) (Figure 2). Additionally, the antimicrobial susceptibility profile was performed by determination of the minimum inhibition concentration (MIC) using the automated VITEK® 2 Compact (bioMérieux®). The strain showed sensitivity to ampicillin (MIC ≤ 2.0 µ / mL), ertapenem (MIC ≤ 0.5 µ / ml), imipenem (MIC ≤ 0.25 µ / ml), meropenem (MIC ≤ 0.25 µ / ml), ciprofloxacin (MIC ≤ 0.25 µ / ml), tigecycline (MIC ≤ 0.5 µ / ml), ampicillin-sulbactam (MIC ≤ 2.0 µ / ml) and piperacillin-tazobactam (MIC ≤ 4.0 µ / ml). Patient data were discussed with the professionals involved and the treatment was initiated with ciprofloxacin, allowing progressive improvement of the infectious condition.

Discussion

In the present study, we report a case of typhoid fever in a young resident in the city of Macapá, in the North region of Brazil, largely covered by the Amazon Forest. The fact that he was infected by S. Typhi endorses data from the Ministry of Health, which indicates that the Brazilian Amazon, comprising the North, Northeast and Midwest regions, is responsible for more than half of the cases of typhoid fever reported nationwide during the last years (13). Macapá is an endemic area where the contamination of rivers is favored by the phenomenon of tides, influenced by the surf and precarious conditions of basic sanitation. A flood was reported days before the patient had symptoms, a fact that may be directly relationship to the infection due to the higher probability of contamination by sanitary sewers.

Figure 2: MALDI-TOF MS spectrum



Global estimates of typhoid fever burden range between 11 and 21 million cases and approximately 128,000 to 161,000 deaths annually (14). Between the years 2016 and 2020, there were 300 confirmed cases in Brazil, with 235 attributed only to the North and Northeast regions. The years 2017 and 2018 had the highest numbers of cases, respectively 113 and 86. The South region has traditionally presented the lowest number of cases and for the Southeast region, the State of São Paulo has presented the highest rates, which may have a direct correlation with the basic sanitation conditions in the peripheries of large urban centers (Table 1).

Table 1. Confirmed cases of Typhoid Fever reported in the information system for notifiable diseases in Brazil

Region (Total of cases) / State of notification	Notification period (year)					
	2016	2017	2018	2019	2020	Total
North region	2	82	48	50	21	203
Rondônia	-	-	1	1	-	2
Acre	-	1	-	1	-	2
Amazonas	-	7	7	5	-	19
Pará	1	38	25	39	3	106
Amapá	1	36	15	4	18	74
Northeast region	(-)	6	19	4	3	32
Piauí	-	-	1	-	-	1
Ceará	-	1	1	1	1	4
Rio Grande do Norte	-	-	1	-	-	1
Paraíba	-	1	-	-	-	1
Pernambuco	-	2	-	1	1	4
Alagoas	-	2	-	1	-	3
Bahia	-	-	16	1	1	18
Southeast region	(-)	25	18	12	7	62
Minas Gerais	-	2	3	1	-	6
Espírito Santo	-	1	1	2	1	5
Rio de Janeiro	-	2	3	1	-	6
São Paulo	-	20	11	8	6	45
South region	(-)	(-)	1	(-)	(-)	1
Rio Grande do Sul	-	-	1	-	-	1
Midwest region	(-)	(-)	(-)	2	(-)	2
Mato Grosso	-	-	-	2	-	2
Total	2	113	86	68	31	300

Source: Ministry of Health/ Health Surveillance Department- Information System for Notifiable Diseases - Sinan Net

It is important to mention that Brazil has made advances in public health issues in recent decades, such as the expansion of the population's access to primary and emergency care, prenatal care and the universalization of vaccination [15]. However, the challenges remain and basic sanitation has a preponderant role, especially when public policies related to this area have peculiarities, as in the case of the countries of Latin America.

Stands out typhoid fever, an acute bacterial disease of high pathogenic potential, of worldwide dissemination and extreme poverty related. In the northern region of Brazil, there are high average annual rates of population growth, an accelerated and uncontrolled growth that is followed by severe socio-environmental problems, mainly related to basic sanitation infrastructures, since many areas are not served by sewage structure and part of the population has no adequate access to the supply of drinking water (7,16-17).

Due to the capacity of dissemination, high infectious potential and high virulence, an accurate and rapid diagnosis is imperative. With this intention, the use of MALDI-TOF MS represents an important assistance, considering its performance concerning agile and efficient identification of species. Conventional methods of identification, such biochemical tests reading, present in modified Rugai medium, may not be effective and could even lead to a misdiagnosis, given the fact that the result for a strain of S. Typhi could easily be interpreted as another Enterobacteriacae. Although the identification of S. Typhi can also be confirmed by the detection of the invA gene by Polymerase Chain Reaction (19), molecular biology techniques tend to be more expensive and are not always widely accessible, especially when diagnostic laboratories or health centers are small and/or located in non-metropolitan regions.

One study analyzed data on more than 10,000 confirmed cases of typhoid incluiding bacterial growth in blood cultures and suggest that outpatient or hospital care was necessary (14). In the present study, the use of the BACT/ALERT® 3D (bioMérieux®) system allowed the indication of positivity with only 4 hours of incubation of both vials of clinical samples. This is a clear advantage over manual methodologies for blood cultures, as already reported by another study (19). Thus, given the use of automated systems in the present study for the detection and identification of microorganisms, the rapid and accurate diagnosis allowed compulsory notification and treatment to be properly performed.

The non-sensitivity of human pathogens to drugs is associated to increased length of stay in a hospital environment and treatment costs, as well as morbidity and mortality. This situation is even more worrying when resources are limited, the disease burden is high and effective antimicrobials are not available or have a high acquisition cost (20).

The treatment for S. Typhi infections often involves the use of ampicillin, chloramphenicol, clotrimazole and fluoroquinolones. However, prior studies have been reported the possibility of therapeutic failure with these antimicrobials. Particularly, in South Asia, fluoroquinolones do not show good efficacy, which requires other options such as cefixime, azithromycin and ceftriaxone, depending on the degree of resistance of the strain (21). In some cases, as already reported in Pakistan (22), the only options are limited to azithromycin and carbapenemics, which can be alarming, as treatment with an intravenous drug, especially in regions with a precarious health system, they may not be accessible or not available. In some Asian countries, ciprofloxacin resistance is close to 100% (23).Studies have already shown high rates, over 90%, of non-sensitivity to fluoroquinolones, as well as an increasing non-susceptibility to other antibiotics such as ceftriaxone, drastically limiting treatment options (20).

Presently, the clinical strain of S. Typhi was found expressing a multi-drug sensitive (MDS) profile. The sensitivity to ciprofloxacin allowed the use of this antimicrobial for patient's treatment and, thus, the clinical improvement and resolution of the infection. Although we reported the isolation of a MDS strain, resistance to three or more antibiotics have already been reported (24), as well as other extensively resistant ones (25). Considering the possibility of an inadequate treatment and the general conditions of health systems and sanitary conditions, as observed in some areas of Brazil and other Latin American countries, we emphasize the importance to carry out the antimicrobial susceptibility test for S. Typhi clinical strains.

In conclusion, the prognosis of the presented case was clearly favored using faster and more assertive microbiological techniques, such as MALDI-TOF MS and automated blood culture, a reality not always widely present in diagnostic laboratories, which suggests that the isolation and identification of S. Typhi should be a concern of the professionals involved, especially in Latin American countries where such infection represents a public health problem. A rapid and adequate treatment is of great relevance, both from the patient's point of view, as to minimize the spread of multidrug-resistant strains and the consequent reduction in therapeutic options, especially in Latin American countries that traditionally face policy problems and access to public health.

Authors' contribution

MRBA: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, and Writting-review & editing. LFS: Data curation, Formal analysis, Investigation and Writting-review & editing. LOS: Data curation, Formal analysis, Investigation, and Writting-review & editing. LSS: Formal analysis, Investigation, Methodology, Project administration, and Writting-review & editing.

Conflict of interest

The authors declare that they have no conflict of interest.

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Ethical statements

Since the study was a retrospective analysis of laboratory data collected and no additional investigations were performed with submitted specimen, no ethical approval was applied.

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