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Epidemiology of *Staphylococcus aureus* infections in Timor-Leste, January–July 2020

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Abstract

Background

The nation of Timor-Leste has a significant burden of infectious disease but has historically had limited diagnostic capacity and limited availability of microbiology data on human health. Recent developments in the diagnosis and reporting of key pathogens including *Staphylococcus aureus* have allowed better understanding of the burden of key infectious diseases and their impact on the population of Timor-Leste.

Methods

A prospective observational study was performed on clinical isolates of *S. aureus* received at Laboratório da Saúde in Dili, Timor-Leste between January 2020 and July 2020. Clinical samples were obtained from patients living in 11 of the 13 municipalities in Timor-Leste. Standard microbiology culture, identification, and antimicrobial susceptibility testing were performed, and clinical and demographic data were collected on laboratory-confirmed *S. aureus* isolates.

Results

A total of 59 clinical isolates of *S. aureus* were identified. Most patients in our study were found to have community-acquired *S. aureus* (75%), whilst the remaining 25% were hospital-associated infections. Of *S. aureus* isolates, 25% were found to be methicillin-resistant.

Conclusion

This is the first description of *S. aureus* infections in Timor-Leste. The high MRSA rates identified in this study can be used to better inform guidelines for the empirical treatment of *S. aureus* infection. Continuous investment in detecting clinically important pathogens and understanding their susceptibility profiles is critical for the development of treatment guidelines and antibiotic stewardship activities.

Keywords: *Staphylococcus aureus*; MSSA; MRSA; antibiotic resistance; Timor-Leste

Introduction

Antimicrobial resistance (AMR) poses a major threat to human health globally. It is a leading cause of death around the world, with 4·95 million deaths associated with bacterial AMR in 2019.[1] AMR affects all countries, but the burden is disproportionately higher in low- and middle-income countries (LMIC).[2] Contributing factors include widespread use of antibiotics, a lack of investment in diagnostic microbiology,[3,4] and limited access to clean water and sanitation.[5] In addition, there are significant data gaps in many low-income settings, emphasising the need to expand microbiology laboratory capacity and AMR surveillance, to better understand the magnitude of the AMR threat and to develop programs to reduce the impact.[1]

Timor-Leste is a resource-poor country located in Southeast Asia with a population of 1.3 million and a high burden of infectious disease. Due to previously limited laboratory capacity to identify infections and determine antimicrobial susceptibility, the AMR burden is not well described. The recent establishment of microbiological diagnostic capacity has permitted substantial improvement in both diagnosis and understanding of the antibiotic susceptibility profiles of key clinically relevant pathogens.[6,7] To date, there is one published study of urine, skin, and soft tissue infection in Timor-Leste, from patients presenting to the Hospital Nacional Guido Valadares (HNGV) in Dili.[8] This prior study showed a moderately high proportion of *Klebsiella pneumoniae* (56%; n = 10/18) and *Escherichia coli* (29%; n = 14/49) isolates with an extended-spectrum β-lactamase (ESBL)-producing phenotype, and a relatively low proportion (11%; n = 4/35) of methicillin-resistant *Staphylococcus aureus* (MRSA).[8] There is currently no published evidence describing the AMR impact in patients from other geographical locations of Timor-Leste, or for other sites of infection. This study aims to describe the epidemiology and antibiotic susceptibility of patients with *S. aureus* infection across 11 of the 13 municipalities of Timor-Leste.

Methods

Study population and sample criteria

This prospective observational study was designed to collect all clinically relevant and laboratory-confirmed *S. aureus* isolates from the Laboratório da Saúde (LdS), Timor-Leste, between January 2020 and July 2020. Samples were collected from both inpatients and outpatients. Outpatients were classified as patients from HNGV outpatient departments; from private clinics; from community health centers (CHCs); and from five referral hospitals in Timor-Leste. Inpatients were classified as patients admitted to HNGV at the time of sample collection. Where patients had more than one isolate culture positive for *S. aureus* (within the same two-week period), only one isolate per patient was included, with the sterile site sample included in preference where applicable.

Definitions

Hospital-associated infection (HAI) was defined as an infection occurring 48 hours or more after admission to HNGV (inpatients) or to another health care facility. In cases where the isolate was grown from a sample collected within 48 hours of hospital admission, these were considered community-acquired infection (CAI). Urine infection was defined as a growth of $> 10^6$ colony forming units per millilitre.

Bloodstream infection was defined as a positive blood culture in bottles that grew *S. aureus*. Methicillin resistance was assessed through disc diffusion using cefoxitin 30 µg, with a diameter of ≤ 21 mm defining MRSA and a diameter of > 21 mm defining methicillin-sensitive *S. aureus* (MSSA). Penicillin-sensitive *S. aureus* (PSSA) was defined as *S. aureus* sensitive to penicillin (1 µg) with a disc of zone size ≥ 26 mm and a fuzzy (or 'beach') zone edge as per EUCAST recommended interpretation guidelines.[9]

Sample processing and identification

All samples received into the Microbiology Laboratory at LdS, including blood, sputum, swabs, mid-stream urine and sterile site fluids, were tested. Swab samples included eye swab, skin, soft tissue, wound or surgical site, and sterile site fluids (pleural fluid, aspirate, cerebrospinal fluid, and swab from brain abscesses). Standard microbiology procedures were performed in adherence to the standard operating procedures (SOPs) at LdS. Samples were inoculated into horse blood agar (HBA), chocolate agar (CA), and chromogenic agar.

All HBA and CA were incubated under 5% CO_2, and chromogenic agar at 37 °C under O_2, for 24–48 hours. Sterile site culture plates were held for five days before discarding. Organisms from non-sterile sites were quantified and read in accordance with Gram stain interpretation. Phenotypic identification of *S. aureus* was performed by morphological reading of agar plates, Gram staining, catalase test and tube coagulase test (Remel), and confirmed using Staph latex test (Oxoid) and BD Phoenix™ M50 PID panels (Becton Dickinson, United Kingdom).

Antibiotic susceptibility testing (AST) for confirmed isolates was performed using both disc diffusion and automated BD Phoenix™ M50 PMIC-84. Antimicrobial interpretation was performed according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (v.10; 2020 guideline);[9] *S. aureus* was classified as penicillin-sensitive *S. aureus* (PSSA), methicillin-sensitive *S. aureus* (MSSA), or methicillin-resistant *S. aureus* (MRSA).

Data analysis

Deidentified demographic and clinical data from all confirmed *S. aureus* isolates were recorded and data were analyzed using χ^2 or Fisher's exact test.

Results

During the six-month study period, a total of 59 *S. aureus* isolates were identified. Most *S. aureus* isolates were MSSA (75%; n = 44/59); the remaining 25% (n = 15/59) were MRSA (Table 1). No PSSA isolates were identified. *S. aureus* isolates were detected from samples received from 11 out of 13 municipalities in Timor-Leste, excluding Viqueque and Região Administrativo Especial Oecusse - Ambeno (RAEOA).

Almost half of the *S. aureus* isolates were from paediatric patients aged 0–10 years and samples were more common from males than females (Table 1). Most isolates (48/59; 81%) came from surface or pus swabs; 9/59 (15%) were from blood culture. Over 90% of the samples were inpatients from the capital city, Dili. CAI was more common than HAI for both MSSA isolates (34/44; 77%) and MRSA isolates (10/15; 67%).

Table 1: Characteristics of methicillin-sensitive (MSSA) and methicillin-resistant *S. aureus* (MRSA) patients in Timor-Leste, January–June 2020

Characteristic	Category	MSSA (N = 44)	MRSA (N = 15)
		n (%)	n (%)
Sex	Male	25 (57)	11 (73)
	Female	19 (43)	4 (27)
Age group (years)	0–10	27 (61)	5 (33)
	11–20	2 (5)	1(7)
	21–30	5 (11)	1 (7)
	31–40	3 (7)	1 (7)
	41–50	3 (7)	0 (0)
	51–60	3 (7)	5 (33)
	> 61	1 (2)	2 (13)
Sample type	Blood	6 (14)	3 (20)
	Swab	36 (82)	12 (80)
	Aspirate	2 (5)	0 (0)
Municipality	Dili	24 (55)	11 (73)
	Manatuto	2 (5)	0 (0)
	Baucau	1 (2)	0 (0)
	Lautem	1 (2)	2 (13)
	Aileu	4 (9)	1 (7)
	Manufahi	3 (7)	0 (0)
	Ainaro	1 (2)	0 (0)
	Covalima	1 (2)	0 (0)
	Bobonaro	1 (2)	1 (7)
	Ermera	4 (9)	0 (0)
	Liquiça	2 (5)	0 (0)
Admission status	Inpatient	40 (91)	14 (93)
	Outpatient	4 (9)	1 (7)
Infection acquisition	Community	34 (77)	10 (67)
	Hospital	10 (23)	5 (23)

Table 2: Clinical characteristics of methicillin-sensitive (MSSA) and methicillin-resistant *S. aureus* (MRSA) patients in Timor-Leste, January–June 2020

Characteristic	Category	MSSA (N = 44) n (%)	MRSA (N = 15) n (%)
Principal diagnosis	Skin and soft tissue infection	35 (80)	9 (60)
	Osteomyelitis	1 (2)	0 (0)
	Pneumonia/ Bronchiolitis	1 (2)	1 (7)
	Conjunctivitis	1 (2)	1 (7)
	Urinary tract infection	0 (0)	1 (7)
	Central nervous system involvement	2 (5)	0 (0)
	Sepsis	1 (2)	2 (13)
	Diabetes	1 (2)	1 (7)
	Immunocompromise	2 (5)	0 (0)
Discharge status	Alive	32 (73)	10 (67)
	Dead	4 (9)	1 (7)
	Discharged against medical advice	1 (2)	1 (7)
	Transferred to another hospital	0 (0)	1 (7)
	Unknown	7 (16)	2 (13)

Clinical characteristics

The most common clinical characteristic of patients in this study was a diagnosis of skin and soft tissue infection (SSTI) (75%; n = 44) (Table 2). Of these, 80% (n = 35/44) had MSSA infection, whilst the remaining 20% (n = 9/44) had MRSA infection. Seventy-three percent of patients with MSSA (n = 32/44) and 67% of patients with an MRSA infection (n = 10/15) were discharged alive; four patients with MSSA and one patient with MRSA died in hospital; the clinical outcome was unknown for nine cases (15%; Table 2). A prolonged hospital stay of more than two weeks was documented for 12/59 cases (20%): these included one blood culture, ten swabs and one aspirate. Seven of these 12 prolonged-stay cases were HAI. There were no statistically significant differences in clinical syndrome distribution between MSSA and MRSA isolates (Fisher's exact $p > 0.10$), with both groups predominantly presenting as a skin and soft tissue infection. Discharge outcomes were also similar between groups, with no significant association observed (Fisher's exact $p > 0.60$). Overall, the clinical presentation and outcomes of *S. aureus* infections in this cohort did not differ meaningfully by methicillin resistance status. Clinical data were incomplete for 21/59 (36%) cases, due to missing data or unclear handwriting.

All 44 MSSA and 15 MRSA isolates were tested for antibiotic sensitivity, and all were resistant to penicillin (Table 3). Of the 44 MSSA isolates, all were sensitive to ciprofloxacin, daptomycin, fusidic acid, teicoplanin, linezolid and vancomycin. High rates of susceptibility to clindamycin (98%), erythromycin (98%), cotrimoxazole (93%) and gentamicin (89%) were observed. Reduced sensitivity of MSSA to tetracycline (64%) was detected.

MRSA isolates showed a different profile of antibiotic sensitivity. All the MRSA isolates were sensitive to ciprofloxacin, cotrimoxazole, daptomycin, fusidic acid, teicoplanin, linezolid and vancomycin. The rates of susceptibility were lower for clindamycin (87%), erythromycin (87%), gentamicin (80%) and tetracycline (87%).

Table 3: Susceptibility of methicillin-sensitive (MSSA) and methicillin-resistant *S. aureus* (MRSA) isolated from infections in Timor-Leste, January–June 2020

	MSSA (N = 44)	MRSA (N = 15)
Antibiotic	n (%)	n (%)
Penicillin	0 (0)	0 (0%)
Cloxacillin	44 (100)	15 (100)
Ciprofloxacin	44 (100)	15 (100)
Clindamycin	43 (98)	13 (87)
Cotrimoxazole	41 (93)	15 (100)
Daptomycin	44 (100)	15 (100)
Erythromycin	43 (98)	13 (87)
Fusidic acid	44 (100)	15 (100)
Gentamicin	39 (89)	12 (80)
Linezolid	44 (100)	15 (100)
Tetracycline	28 (64)	13 (87)
Teicoplanin	44 (100)	15 (100)
Vancomycin	44 (100)	15 (100)

Discussion

In this study, we identified benzylpenicillin resistance in both MSSA and MRSA, while no resistance was recorded to vancomycin, teicoplanin, daptomycin or fusicidic acid. We also found a low level of co-resistance. All 15 MRSA isolates were susceptible to vancomycin, teicoplanin, fusidic acid, ciprofloxacin, daptomycin and cotrimoxazole. For MSSA, cloxacillin and/or clindamycin is effective to cover the infection, whilst for MRSA the first-line treatment can be cotrimoxazole or tetracycline, with vancomycin as the last line, though this depends on the site of infection. Vancomycin is the first-line intravenous treatment for MRSA infections in Timor-Leste, and cotrimoxazole is likely to be a good oral option, especially in cases with clindamycin resistance. Linezolid, daptomycin and teicoplanin are not on the essential medicines list in Timor-Leste and are not routinely available. Ongoing vigilance is required, given the potential for emergence of further resistance, including to vancomycin.[10]

Our study of *S. aureus* infection in Timor-Leste, including patients from 11 of the country's 13 municipalities and with representation of a greater variety of sample types, is larger than the only previous study by Marr et al.[8] While the MRSA rate identified in this study (25%; 15/59) is higher than that described by Marr et al. (11%; 4/35), both studies had a small sample size; accordingly, the difference in MRSA rates may be attributed to random error. There were four deaths recorded in this study; however, limited clinical data hampered the elucidation of any correlation between the type of *S. aureus* infection and the clinical outcome.

Most of our samples collected were from the capital city, Dili, where the recently established diagnostic microbiology laboratory is located. Prior to this, Timor-Leste had limited capacity for diagnostic microbiology. High investment in capacity building and training local scientists has ensured a well-established diagnostic microbiology laboratory which services the entire country, with a commitment to expand to more referral hospitals in other municipalities in upcoming years.[6,11]

Limitations

This study has several limitations, some of which relate to the manual nature of data collection and record keeping. Missing data were common in Timor-Leste, due to manual data collection in all levels of clinical care. With the implementation of the Laboratory Management Information System at the LdS in September 2020, this issue is likely to be resolved for future studies.[11] In addition, there were smaller than expected numbers of patients attending outpatient consultation clinics, due to public health restrictions in the country and high coronavirus disease 2019 (COVID-19) community transmission during the study period.

Conclusion

Our findings suggest a high rate of MRSA in clinical isolates in Timor-Leste and support the ongoing necessity of high quality AMR surveillance, and linkage of laboratory findings with clinical outcomes, to better understand the epidemiology and impact of AMR across the country. These actions are needed to support local antimicrobial stewardship efforts to reduce the high burden of AMR in Timor-Leste.

Funding statement

No funding was provided for this study.

Conflict of interest

The authors declare no conflict of interest regarding the publication of this article.

Ethics approval

Ethical approval was obtained from Instituto Nacional de Saúde (INS) Timor-Leste (Reference number: 70 MS-INS/DE/I/2020).

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