

## Research paper

# The application of environmental health assessment strategies to detect *Streptococcus pyogenes* in Kimberley school classrooms

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## KEYWORDS

*Streptococcus pyogenes*;  
 Aboriginal and Torres Strait Islander health;  
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 Transmission

**Abstract** *Background:* Children spend almost one-third of their waking hours at school. *Streptococcus pyogenes* (Strep A) is a common childhood bacterial infection that can progress to causing serious disease. We aimed to detect Strep A in classrooms by using environmental settle plates and swabbing of high-touch surfaces in two remote schools in the Kimberley, Western Australia.

*Methods:* Twelve classrooms in two schools participated in 2021 and 2022. Seven horse-blood agar plates containing colistin and nalidixic acid (HBA-CNA) were placed in each occupied classroom at varied heights for 4 h, and 20 high-touch items were swabbed and later cultured on HBA-CNA plates. The primary outcome of each sample was presence or absence of Strep A. Identified Strep A isolates were whole genome sequenced (WGS) to assess for similarity between host-derived and environmental strains.

*Results:* During two visits to each participating Kimberley school in June 2021 and September 2022, the point prevalence of Strep A positive throat swab ranged between 3/34 (8.8 %) and 5/21 (23.8 %); Strep A impetigo was lower at between 0/43 (0 %) and 2/23 (8.7 %). Strep A was detected from 4/240 (2 %) environmental swabs collected across 3/12 (25 %) classrooms but not cultured from any of the classroom settle plates. Whole genome sequencing identified

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environmental *emm* types to also be those strains in circulation.

**Conclusions:** There was little evidence to support fomite, droplet or airborne Strep A in classrooms as major modes of transmission among children. Further work is required to determine if classrooms play a role in the transmission of Strep A between students.

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### Highlights

- Schools present an important setting for Strep A transmission.
- We did not detect Strep A in classrooms using settle plates.
- Fomite transmission potential is possible with 4 objects positive for Strep A.

## Introduction

*Streptococcus pyogenes* (Strep A) is a human only pathogen [1] with an array of clinical phenotypes [2]. It is among the most common pathogenic infections isolated from children and while many infections resolve quickly without antibiotics, some will progress to life-threatening or serious downstream sequelae including acute rheumatic fever (ARF) and rheumatic heart disease (RHD) [3]. The predominant transmission mode is attributed to large respiratory droplets [4–7] although emerging research has implicated airborne (via small droplets), skin-to-skin contact, contact with fabrics, surfaces and foodborne transmission modalities [8].

Australia has one of the highest reported rates of ARF and RHD in the world, concentrated among Aboriginal and Torres Strait Islander populations and communities – mostly in the northern regions of Australia – where access to culturally appropriate and accessible healthcare is limited [9]. In addition, the acquisition of ARF and progression to RHD is driven by repeated exposure to Strep A [10], greatest in prevalence in regions with sparse or inadequate health hardware or overcrowded homes [11]. In essence, the high burden of ARF and RHD in Australia cannot be reduced without a concurrent reduction of Strep A infection in the population most vulnerable to these downstream illnesses.

The RHD Endgame Strategy, published in 2020, provided a blueprint to the elimination of RHD in Australia by 2031 [12]. Specifically, the report highlighted a need for further research to reduce the transmission of Strep A, referred to as primordial prevention strategies. To date, most research into primordial prevention of Strep A has centred on the home environment. However, given the time spent by children at school (approximately 30 h of a week ~a third of time spent awake), there is relevance to increase our understanding of the potential for Strep A transmission schools.

Current Australian guidelines recommend that students with Strep A impetigo or pharyngitis are excluded from school until 24-h post commencement of antibiotic treatment [13]. Whilst this is an important public health containment strategy, these guidelines may have unintended consequences on educational outcomes specifically

in the context of recurrent infections or settings where school attendance averages are 70 % [14]. Improved information is required.

This sub-study was a component of The Missing Piece Surveillance Study [15] aiming to evaluate the concurrent burden of Strep A impetigo and pharyngitis in Aboriginal children. It is expected that results garnered from this project will assist with the development of more specific recommendations for Strep A disease treatment and prevention. As such, a better understanding of the likelihood of transmission in settings where the population of highest incidence of ARF – children aged 5–15 years – spend a significant portion of their day is required. Therefore, this sub-study aimed to determine if Strep A can be detected in a remote school setting with a high burden of Strep A infection. Three specific modes of transmission were investigated; fomites via the swabbing of high touch surfaces; large respiratory droplets with the placement of environmental settle plates below a metre high and small respiratory droplets (airborne) with settle plate placement above 2 m.

## Methods

### Setting

The Missing Piece Surveillance Study [15] occurred between 2019 and 2022 in two schools the Kimberley region of Western Australia. The Kimberley encompasses 423,517 square kilometres (km [2]) of the State's north with a population of 35,000 residents [16]. Of these, approximately 41 % identify as Indigenous [16]. Inadequate access to household hygiene infrastructure across the Kimberley is common and a driver of repeated Strep A infections [9]. Kimberley schools are externally managed either by Catholic Education Western Australia, the State Department of Education or Independently [9] and generally have a classroom size of between 20 and 32 students, dependant on age [17].

In brief, the Missing Piece Surveillance Study comprised of two specific components; screening of healthy and symptomatic children at school for pharyngitis and impetigo up to three times a year and weekly active surveillance visits to detect new symptomatic cases of pharyngitis and impetigo between 2019 and 2022 (with interruptions to this

schedule caused by COVID-19) [15]. This environmental health sub-study was conducted and planned to coincide with a screening visit in September 2021 and June 2022 to capitalise on research resources and personnel and data collected during these visits also allowed for the determination of prevalence estimates for Strep A [15]. The Missing Piece Surveillance Study protocol can be viewed elsewhere [15] and research results are forthcoming.

### Classroom observation

To explore possible transmission via fomites, droplet or airborne routes, three classrooms at each school were selected for environmental assessment, prioritising classes with students aged 5–9 years. Selection was guided by conversations with the school Principal as to which students would be utilising their classroom most (i.e., avoiding school excursions, sport or library use) and who would be disrupted less by researchers entering their classroom throughout the day. For selected classrooms, two researchers observed students within their classroom for 20 min to quantify the most high-touched items and/or surfaces. Items touched were noted and subsequent tallies made. Observations were combined in Microsoft Excel, and items ranked from most to least touched. Fifteen unique items were selected in each classroom to be swabbed, as well as the following 'out of reach' areas; an air vent and the top of a window frame, window, whiteboard and cupboard. Where appropriate, discussions with classes explained the research aims and elicited student input and suggestions as to items swabbed.

### Settle plate placement (investigation of airborne and droplet transmission routes)

Horse-blood agar plates containing colistin and nalidixic acid (HBA-CNA) plates (PathWest, Australia; 90 mm diameter) were chosen to optimise the detection of Strep A and minimise the growth of environmental gram-negative organisms. For each classroom, four plates were placed on removable adhesive shelves at a height of 2.1 m on each wall to detect possible Strep A airborne spread. Following consultation with teachers to discern the most appropriate location – ensuring plates were out of the way of normal classroom activities and could not be touched by students – an additional three plates were placed on accessible surfaces to identify possible droplet spread at an average height of 82 cm. At the request of teachers, these lower plates were out of reach of children. Plates were exposed for 4 h in the classroom, with times selected in negotiation with classroom schedules to maximise the time students were indoors. Each classroom had a wall mounted air conditioner that was operational during the study period.

### Environmental swabbing (investigation of fomite transmission routes)

FLOQ swabs moistened with sterile saline were used to swab an area of approximately 25 cm<sup>2</sup> of the twenty 'identified' and 'pre-determined' high-touch items/surfaces. Swabbing occurred at the end of the school day and

each swab was rubbed over the surface for up to 10 s in three alternate directions before any potential classroom cleaning. Swabs were placed immediately in SGGB at refrigeration temperature (4–8 °C) prior to and during transport subsequent to storage at –80 °C and culture in batches at Telethon Kids Institute in Perth, Western Australia.

### Culture and identification of Strep A presence

HBA-CNA settle plates exposed in the classroom were transported on the day of collection at refrigeration temperature (4–8 °C) to nearby laboratories in Derby and Broome (PathWest) and incubated for 24 h at 35 °C. Positive and negative control plates were incubated for quality assurance. If no growth of  $\beta$ haemolytic colonies was detected after this time, incubation for an additional 24 h occurred. Any beta-haemolytic colonies were tested with Strep latex agglutination kits as previously described. The swabs in SGGB were transported to Perth at 4 °C and frozen at –80 °C until being cultured in batches. They were defrosted, vortexed and a 20  $\mu$ L sample of transport medium streaked onto HBA-CNA plates for single colonies as per standard procedures. Plates were incubated and assessed using the same methods as described above. The primary outcome for each sample was the presence or absence of Strep A. Other microbes present on any items were not worked up further to identify them.

### Whole genome sequencing

Whole genome sequencing (WGS) was performed to fully characterise the relatedness of Strep A isolates cultured. This process also allowed for a comparison of Strep A collected from symptomatic and asymptomatic participants and the environment. Strep A isolates were cultured from frozen stocks stored in glycerol and cell lysates prepared using phage lysis [18]. Genomic DNA was extracted from lysates using the DNeasy Blood and Tissue kit (Qiagen 69504) according to the manufacturer's instructions. Extracted DNA was sequenced on the Illumina NovaSeq platform with paired 150 bp chemistry (Australian Genome Reference Facility, Australia). All genomic data was analysed using the Bohra analysis pipeline ver. 2.3.2 (<https://github.com/MDU-PHL/bohra>). Briefly, raw reads were filtered for quality and species identity, assembled using shovill ver. 1.1.0, and annotated with prokka. The pipeline was also used to determine the MLST type (mlst ver. 2.23.0) and *emm* type (emmtyper ver. 0.2.0). Core genome single nucleotide polymorphisms (SNPs) were detected with snippy ver. 4.4.5 to determine the relatedness of GAS isolates from environmental sources and throat swabs.

### Ethics and approvals

This project was approved by the Western Australian Aboriginal Health Ethics Committee (REF-892) and the University of Western Australia (RA/4/20/5101), Catholic Education WA (REF: RP2018/55), the Kimberley Aboriginal

**Table 1** Strep A burden in classrooms combining child and environmental results at same time points.

Year	2021			2022					
Classroom	1	2	3	7	8	9	10	11	12
School	1			1			2		
Enrolled in class	17	17	22	16	10	14	18	25	32
Recruited to study	5 (29.4 %)	9 (53.0 %)	13 (59.0 %)	1 (6.3 %)	4 (40.0 %)	3 (21.4 %)	4 (22.2 %)	5 (20.0 %)	10 (31.3 %)
Present during screening week	4 (80.0 %)	9 (100 %)	12 (92.3 %)	1 (100 %)	4 (100 %)	1 (33.3 %)	1 (25.0 %)	3 (60.0 %)	10 (100 %)
Throat swab taken	3 (75/0 %)	7 (77.8 %)	9 (75.0 %)	1 (100 %)	3 (75.0 %)	1 (100 %)	0 (0 %)	3 (100 %)	8 (80.0 %)
Strep A+ throat swab and <i>emm</i> type	0 (0 %)	0 (0 %)	1 (11.1 %)	0 (0 %)	0 (0 %)	0 (0 %)	N/A	0 (0 %)	1 (12.5 %) ST1108: <i>emm</i> 114
Skin swab taken	1 (20.0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	1	1 (100 %)	1 (33.3 %)	2 (20.0 %)
Strep A+ skin swab	0 (0 %)	N/A	N/A	N/A	N/A	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
Strep A + EH swabs (n = 20 in each classroom) and <i>emm</i> type	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	1 (5 %) (ST140: <i>emm</i> 114)	2 (10 %) ST182: <i>emm</i> 101, ST392: <i>emm</i> 103	1 (5 %) (ST182: <i>emm</i> 101)
Point prevalence across screening visit (whole school)	Strep A + throat swab 3/34 (8.8 %)			5/21 (23.8 %)			6/65 (9.2 %)		
	Strep A + impetigo 0/43 (0 %)			2/23 (8.7 %)			0/71 (0 %)		

Health Planning Forum Research Subcommittee and Principals from each school involved in the project.

## Results

### Prevalence of Strep A among children

During two visits to each participating Kimberley school (June 2021 and September 2022), the point prevalence of Strep A positive throat swab ranged from 3/34 (8.8 %) to 5/21 (23.8 %); Strep A impetigo was lower at between 0/43 (0 %) and 2/23 (8.7 %, [Table 1](#)).

Among 9 participating classrooms, 171 were enrolled. Enrolment data was unavailable for 3 classrooms during 2021 ([Fig. 1](#)). Attendance for each day was not collected as not all children present at school had consented to pharyngitis and impetigo surveillance. 53 of the 171 students enrolled in the school were participants in the Missing Piece Study (30.9 %), and 44 (83.0 %) were present. Of these, 36 (81.8 %) consented to having a throat swab taken and 4 (9.1 %) had a skin sore swabbed. Only 2 of the throat swabs were Strep A positive (one each in School 1, 2021 and School 2, 2022) and no skin sores were positive for Strep A ([Fig. 1](#), [Table 1](#)).

### Frequency of Strep A detection from frequently touched surfaces and settle plate

A total of 84 plates and 240 swabs were collected in twelve classrooms at two Kimberley schools in June 2021 and September 2022 visits. From 240 surface swabs ([Tables 2 and 3](#)), four had Strep A detected ([Table 3](#)). These 4 positive environmental surface swabs were collected from 3 classrooms at the same school in June 2022 ([Table 3](#)) when the point prevalence of Strep A positive throat swabs across the school was 6/65 (9.2 %) with no active Strep A impetigo detected. Swabbed items were: a fabric mat that children sat on, a wooden door, a wooden table and a plastic ruler. Strep A was not detected on any of the 84 settle plates (see [Table 1](#)).

### Whole genome sequencing typing results

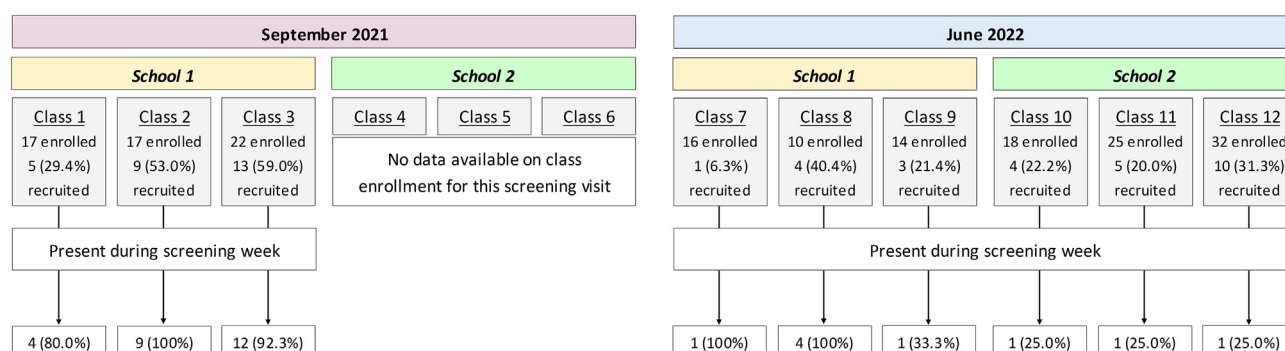
Genome sequencing confirmed that isolates from the 4 positive surface swabs belonged to *emm101*:ST182 (two isolates) *emm114.7*:ST140 and *emm103*:ST392. The two

identified *emm101* isolates, which were isolated from two different classrooms, differed at 312 core genome SNPs. This is indicative of the isolates belonging to two independently circulating genomic lineages. However, *emm101* isolates from the positive EH swab in school 2, classroom 9 in 2022 and a throat swab from a child that same week at school 1 (carriage) differed by only 3 SNPs. This suggests that this genomic lineage was circulating at both schools during this period. The other three Strep A lineages isolated from environmental swabs differed from throat isolates collected during the study by 17 (*emm114.7*), 51 (*emm101*), and 45 (*emm103*) SNPs.

## Discussion

This sub-study embedded environmental health research within a well-established school-based prospective cohort study for Strep A detection, allowing the opportunity to combine primordial and primary prevention research in the same setting. We determined that while possible to detect Strep A from frequently touched items, this was uncommon. We did not detect evidence of Strep A in droplet or airborne experiments, suggesting it may not be a dominant method of transmission in these settings or unidentifiable using our chosen methods.

The Kimberley region of Western Australia continues to experience a high burden of ARF and RHD; diseases that are largely preventable and barely existent in non-Indigenous populations across Australia [19]. Eliminating the downstream consequences of Strep A infection is dependent upon an adequate understanding of Strep A transmission in endemic regions, particularly in settings such as schools where the concentration of children most at risk (aged 5–9 years of age) is highest. Our work indicates further research possibly with a different methodology is required to explore the role of droplet transmission of Strep A in Kimberley schools. The role of fomite transmission remains speculative as a dominant transmission modality given only four items had Strep A detected on them. However environmental recovery of Strep A from culture of surface swabs may be low yield unless obtained soon after inoculation from a colonised individual. This consideration informed swabbing classrooms on the same day as children's skin and throats, however small numbers of positive results make it difficult to conclusively exclude fomite transmission [20,21].



**Fig. 1** Numbers of students involved in the Missing Piece Surveillance Study during these environmental health experiments.

**Table 2** Items swabbed in classrooms during September 2021.

School	School 1			School 2		
Classroom	1	2	3	1	2	3
Items swabbed	<ul style="list-style-type: none"> <li>• Wooden blocks</li> <li>• Paint table</li> <li>• Wooden stationary table</li> <li>• Group desk</li> <li>• Wooden bookshelf</li> <li>• Bench</li> <li>• Lego table</li> <li>• Side of bin</li> <li>• Play phone</li> <li>• Play kitchen</li> <li>• Door handle (metal)</li> <li>• Reading frame (wooden)</li> <li>• Plastic chair</li> <li>• Plastic chair (2)</li> <li>• Door frame (wooden)</li> <li>• Air vent</li> <li>• Windowsill</li> <li>• Top of window</li> <li>• Top of whiteboard</li> <li>• Top of cupboard</li> </ul>	<ul style="list-style-type: none"> <li>• Fridge handle</li> <li>• Door handle (metal)</li> <li>• Classroom mat</li> <li>• A4 whiteboard</li> <li>• A4 whiteboard (2)</li> <li>• Group desk</li> <li>• Marker box</li> <li>• Water tap</li> <li>• Group desk (2)</li> <li>• Group desk (3)</li> <li>• Group desk (4)</li> <li>• Plastic chair</li> <li>• Plastic chair (2)</li> <li>• Light switch</li> <li>• Bookshelf</li> <li>• Laminating table</li> <li>• Windowsill</li> <li>• Top of window</li> <li>• Top of whiteboard</li> <li>• Top of cupboard</li> </ul>	<ul style="list-style-type: none"> <li>• Doorknob (metal)</li> <li>• Water tap</li> <li>• Calculator</li> <li>• Plastic chair</li> <li>• Plastic stool</li> <li>• Group desk</li> <li>• Group desk (2)</li> <li>• Group desk (3)</li> <li>• Plastic floor desk</li> <li>• Plastic floor desk (2)</li> <li>• Fan switch</li> <li>• Whiteboard marker</li> <li>• Light switch</li> <li>• Bookshelf</li> <li>• Pigeonhole tray</li> <li>• Air vent (air con)</li> <li>• Windowsill</li> <li>• Top of window</li> <li>• Top of whiteboard</li> <li>• Top of cupboard</li> </ul>	<ul style="list-style-type: none"> <li>• Messy play table</li> <li>• Plastic play bowl</li> <li>• Wooden easel</li> <li>• Paint brush</li> <li>• iPad</li> <li>• Rubber stool</li> <li>• Rubber stool (2)</li> <li>• Paper puppets</li> <li>• Wooden table</li> <li>• Bookshelf</li> <li>• Wooden table (2)</li> <li>• You microwave handle (wooden)</li> <li>• Plastic pencil container</li> <li>• Plastic chair</li> <li>• Plastic chair (2)</li> <li>• Fridge handle</li> <li>• Windowsill</li> <li>• Top of window</li> <li>• Top of whiteboard</li> <li>• Doorhandle (metal)</li> </ul>	<ul style="list-style-type: none"> <li>• Pigeonhole tray</li> <li>• Group table</li> <li>• Group table (2)</li> <li>• Group table (3)</li> <li>• Plastic chair</li> <li>• Plastic chair (2)</li> <li>• Rubber stool</li> <li>• Pencil tin</li> <li>• Mini drawers</li> <li>• Pack up bell</li> <li>• Plastic floor desk</li> <li>• Plastic floor desk (2)</li> <li>• Teacher's chair (wooden)</li> <li>• Fit ball</li> <li>• Doorknob (metal)</li> <li>• Door handle (metal)</li> <li>• Windowsill</li> <li>• Top of window</li> <li>• Top of whiteboard</li> <li>• Top of cupboard</li> </ul>	<ul style="list-style-type: none"> <li>• iPad/Screen</li> <li>• A4 whiteboard</li> <li>• A4 whiteboard (2)</li> <li>• Pigeonhole tray</li> <li>• Pigeonhole tray (2)</li> <li>• Under window shelves</li> <li>• Whiteboard marker</li> <li>• Group table</li> <li>• Coffee table (wooden)</li> <li>• Wooden stool</li> <li>• Group table (2)</li> <li>• Rubber stool</li> <li>• Doorknob (metal)</li> <li>• Desk on wheels</li> <li>• Pencils tins</li> <li>• Doorknob (2, metal)</li> <li>• Windowsill</li> <li>• Top of window</li> <li>• Top of whiteboard</li> <li>• Top of cupboard</li> </ul>

**Table 3** Items swabbed in classrooms during June 2022.

School	School 1			School 2		
	Classroom 1	2	3	1	2	3
Items swabbed	<ul style="list-style-type: none"> <li>• Pigeonhole tray</li> <li>• Plastic chair</li> <li>• Numeracy table</li> <li>• Box of plastic fruits</li> <li>• Abacus</li> <li>• Entry door frame at base of right window</li> <li>• Sunscreen pump</li> <li>• Toy telephone</li> <li>• Wooden puzzle</li> <li>• Plastic table</li> <li>• Cushion</li> <li>• Teacher's chair (fabric)</li> <li>• Water bottle (plastic)</li> <li>• Lego brick</li> <li>• Doorknob (metal)</li> <li>• Windowsill</li> <li>• Top of window</li> <li>• Top of whiteboard</li> <li>• Top of cupboard</li> </ul>	<ul style="list-style-type: none"> <li>• Classroom mat</li> <li>• Fridge door</li> <li>• Fridge handle</li> <li>• Whiteboard eraser</li> <li>• Group desk</li> <li>• Plastic chair</li> <li>• Group desk (2)</li> <li>• Plastic chair (2)</li> <li>• Whiteboard pointer</li> <li>• iPad</li> <li>• Whiteboard</li> <li>• Whiteboard (2)</li> <li>• Reading book</li> <li>• Pigeonhole tray</li> <li>• Doorknob (metal)</li> <li>• Air vent (air con)</li> <li>• Windowsill</li> <li>• Top of window</li> <li>• Top of whiteboard</li> <li>• Top of cupboard</li> </ul>	<ul style="list-style-type: none"> <li>• Cupboard handle (metal)</li> <li>• Telephone buttons (landline)</li> <li>• Headphones</li> <li>• Lead pencil</li> <li>• Plastic chair</li> <li>• Plastic chair (2)</li> <li>• iPad</li> <li>• iPad (2)</li> <li>• Teacher's chair (fabric)</li> <li>• Edges of bench in front of TV</li> <li>• Sink tap</li> <li>• Soap dispenser</li> <li>• Air con remote</li> <li>• Light switch</li> <li>• Doorknob (metal)</li> <li>• Air vent (air con)</li> <li>• Windowsill</li> <li>• Top of window</li> <li>• Top of whiteboard</li> <li>• Top of cupboard</li> </ul>	<ul style="list-style-type: none"> <li>• A4 whiteboards</li> <li>• Reading book</li> <li>• Table (next to sink)</li> <li>• Whiteboard markers</li> <li>• Spelling letters</li> <li>• Fabric mat<sup>a</sup></li> <li>• Couch</li> <li>• Wooden tabletop</li> <li>• Wooden phone</li> <li>• Wooden food</li> <li>• Alphabet charts</li> <li>• Rubber stool</li> <li>• Whiteboard marker holder</li> <li>• Door handle (metal)</li> <li>• Pigeonhole trays</li> <li>• Sink tap</li> <li>• Windowsill</li> <li>• Top of window</li> <li>• Top of whiteboard</li> <li>• Top of cupboard</li> </ul>	<ul style="list-style-type: none"> <li>• Classroom mat</li> <li>• Pigeonhole tray</li> <li>• Wooden tabletop</li> <li>• Plastic chair</li> <li>• Teacher's chair (fabric and wood)</li> <li>• Plastic rub</li> <li>• Water bottle</li> <li>• Whiteboard</li> <li>• Smart TV</li> <li>• Wicker basket</li> <li>• Whiteboard marker</li> <li>• Group desk<sup>a</sup></li> <li>• Stationary bucket</li> <li>• Door (wooden)<sup>a</sup></li> <li>• Doorknob (metal)</li> <li>• Door handle (metal)</li> <li>• Windowsill</li> <li>• Top of window</li> <li>• Top of whiteboard</li> <li>• Top of cupboard</li> </ul>	<ul style="list-style-type: none"> <li>• Stationary tray (metal)</li> <li>• Classroom mat</li> <li>• Fabric toy</li> <li>• Clipboards</li> <li>• Plastic ruler<sup>a</sup></li> <li>• Group desk</li> <li>• Pencil</li> <li>• Texta</li> <li>• Group desk (2)</li> <li>• Plastic chair</li> <li>• Door handle (metal)</li> <li>• Whiteboard eraser</li> <li>• Couch</li> <li>• Teacher's iPad</li> <li>• Door wooden</li> <li>• Tray tables</li> <li>• Windowsill</li> <li>• Top of window</li> <li>• Top of whiteboard</li> <li>• Top of cupboard</li> </ul>

<sup>a</sup> Denotes Strep A was detected on these items.

Whole genome sequencing results demonstrated that the Strep A isolated from objects shared strains with throat swabs collected from students. The closest similarity as discerned by SNP differences was between a strain found on a classroom ruler (plastic) at School 2 and from a swab taken from a child with asymptomatic throat carriage at the School 1 220 km away. We interpret this result as indicating active circulation of the ST182: *emm101* strain at time of sampling despite no swabs taken from students at School 2 displaying similarity. This is especially likely given the relatively low level of swabbing completed during the screening visit comparable to the number of students enrolled in the school. Coverage was approximately 30 % which may have reduced our ability to capture student and environmental strains concurrently even if they were occurring.

Since the COVID-19 pandemic, significant emphasis has been placed on ensuring classrooms are well ventilated to reduce transmission of droplet and airborne-spread contagions among pupils [22]. Classrooms in our study had windows and doors closed with fans and air-conditioners for cooling, yet no HBA-CNA settle plate yielded a positive Strep A result. This suggests environmental conditions were perhaps not favourable for detection of Strep A via our culture method. HEPA filters were installed in all WA classrooms in late 2021, so this may have impacted our results [23]. As four classroom items had Strep A cultured on them, regular cleaning of classroom surfaces and objects with antibacterial methods may be beneficial.

Strep A epidemiology can fluctuate over time due to various environmental factors such as frequency of cleaning, weather conditions, temperature changes, and seasonal variations. Sampling at a single time point may not capture these fluctuations, leading to an incomplete or inaccurate assessment of transmission of Strep A. Additionally, infrequent sampling may fail to capture short-term spikes or outbreaks of Strep A infection. Regular and strategically timed sampling is necessary to develop a comprehensive understanding of transmission dynamics over time. Future planned studies should consider a well-planned sampling strategy that accounts for these fluctuating changes in the environment and includes frequent and strategically timed sampling events.

A strength of this research was the ability to engage students and capitalise upon their familiarity with their classroom environment to guide classroom swabbing strategies. This not only served as an opportunity for science communication and health promotion but allowed for student input, and substantiated evidence to ensure the most high-touched objects were selected, especially given observation only captured a small part of the classroom day. An additional strength was the completion of the environmental assessments concurrently with screening visits, allowing a comparison of the Strep A point prevalence and further investigation using WGS.

This research had several limitations. Several of the settle plates at lower heights (averaging 92 cm) were contaminated by ants (6 in 2021 and 1 in 2022) and a further 6 plates placed at 2.1 m fell off their shelf during the 4-h period. All plates were incubated and read for presence or absence of Strep A despite these challenges. In addition, all classrooms had wall mounted air-conditioners which likely

had affected transmission dynamics. Future research should focus on integrating measurements of temperature, humidity, and airflow. While all classrooms were swabbed at the end of the school day, we were unable to control for surface cleaning that may have occurred during the day nor confidently ascertain the last time the classroom was cleaned and to what extent. Further, despite best attempts to identify high-touch items that could harbour Strep A only a small minority of classroom objects were swabbed, and it is likely some Strep A-contaminated items were not identified. This could be an explanation as to why all items swabbed were negative until the final timepoint. Environmental contaminants may have been impacted by the fact that the study was conducted during a pandemic when significant emphasis was placed on environmental and personal hygiene. Only items *within* classrooms were swabbed and future research may benefit from the inclusion of items such as bathroom taps and shared playground equipment. Lastly, we were not privy to the attendance of each classroom and could therefore not quantify classroom occupancy which would have had merit in better estimating the Strep A burden. Similarly, not all attending children were participating in the study - further reducing our coverage - and we were also unable to discern if children with active Strep A infections had been excluded from school on the day of sampling as a result of public health guidelines.

In conclusion, there was insufficient evidence to determine whether fomite, droplet or airborne modes of transmission contribute to superficial Strep A infections among children at high risk of rheumatic fever. The time, cost and educational impost of these studies are high, and this experience should temper design considerations for further studies to determine whether classrooms play a role in the transmission of Strep A between students.

## Authorship statement

SLE: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Validation; Visualization; Writing – original draft; Writing – review & editing. BW: Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Validation; Writing – review & editing. AM: Data curation; Formal analysis; Investigation; Writing – review & editing. AJF: Investigation. MO: Methodology; Investigation; Formal analysis. TP: Investigation. LB: Project administration; Resources. HMMT: Supervision; Validation; Writing – review & editing. NL: Supervision; Validation; Writing – review & editing. JC: Supervision; Validation; Writing – review & editing. DDB: Investigation; Methodology; Project administration; Resources; Writing – review & editing. JP: Data curation; Formal analysis; Investigation; Methodology; Project administration; Supervision; Validation; Writing – review & editing. ACB: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Resources; Supervision; Validation; Visualization; Writing – review & editing.

## Declaration of Competing Interest

The authors declare no conflicts of interest.

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## Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

## Provenance and peer review

Not commissioned; externally peer reviewed.

## Ethics

This project was approved by the Western Australian Aboriginal Health Ethics Committee (REF-892) and the University of Western Australia (RA/4/20/5101), Catholic Education WA (REF: RP2018/55), the Kimberley Aboriginal Health Planning Forum Research Subcommittee and principals from each school involved in the project.

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