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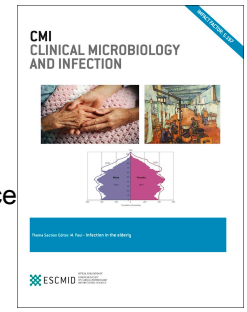

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Investigation of trimethoprim-sulfamethoxazole resistance in an emerging sequence type 5 methicillin-resistant *Staphylococcus aureus* clone reveals discrepant resistance reporting

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Running title: Discrepant SXT resistance reporting of ST5 MRSA clone

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Sir,

In regions where community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) is common, oral trimethoprim sulfamethoxazole (SXT) is a treatment option for skin and soft tissue infections. Recent trials have demonstrated non-inferiority of SXT in comparison to intramuscular benzathine benzylpenicillin [1] or oral clindamycin [2] for treatment of uncomplicated skin and soft tissue infections (SSTI) such as impetigo [1], cellulitis, and simple abscesses [2]. Concern over the increasing rates of community-acquired *S. aureus* isolates reported as SXT-resistant, has resulted in recommendations against prescribing SXT for the treatment of SSTIs in the Kimberley region of Western Australia [3].

During a clinical trial of SXT for treatment of impetigo in northern Australia (referred to as the Skin Sore Trial) [1], 22/2211 *S. aureus* isolates (<1%), recovered from 8/508 children (1.6%) in the trial, were reported as SXT-resistant by Vitek®2 (bioMérieux, France), using the Gram-positive AST-P612 card according to manufacturer's instructions. To confirm SXT resistance, 19 of these isolates were tested for SXT and trimethoprim resistance by Etest® (bioMérieux) according to manufacturer's instructions. All 19 isolates were classed as trimethoprim-resistant but SXT-susceptible by Etest® (Table 1). Two of these isolates were tested independently in a second laboratory and also reported as SXT-resistant by Vitek®2, and trimethoprim-resistant and SXT-susceptible by Etest® (Table 1). Repeat Vitek®2 testing using low thymidine content subculture media did not change the results. These isolates were also susceptible to SXT when tested using the Sensititre® broth microdilution (ThermoScientific, United Kingdom) (Table 1).

Whole genome sequence analysis revealed that the 19 Skin Sore Trial isolates were all sequence type (ST) 5 and harboured *lukS-PV* and *lukF-PV* (encoding for Panton-Valentine leucocidin). Genome alignment to ST5 reference strain Mu50 demonstrated that the isolates are a closely related clone, with a maximum of 15 orthologous single nucleotide polymorphisms (SNPs) and three indels observed between any two isolates.

All 19 isolates harboured a novel SCC*mec*, consisting of a SCC*mec*IVc backbone, with a putative *dfrG* mobile genetic element [4] located immediately upstream of IS*431* in the J3 region. This novel SCC*mec* construct has been assigned as SCC*mecIVo* by the International Working Group on the Staphylococcal Cassette Chromosome Elements. Consistent with the SXT-susceptible phenotype indicated by Etest® and Sensititre®, we were unable to identify any acquired genes or mutations that potentially confer sulfamethoxazole resistance (specifically the absence of acquired sulphonamide resistance genes *sulI*, *sulII* and *sulIII*, and mutations in the chromosomal dihydropteroate synthase gene *folP*).

The discrepancy between the Vitek®2, Etest®, and Sensititre® results was not restricted to a specific clone or ST. An additional 12 clinical *S. aureus* isolates from a separate study, including non-ST5 strains, reported as SXT-resistant by Vitek®2 (MIC 40 - ≥320 µg/mL) were reported SXT-susceptible by Etest® (MIC 0.023 – 2.0 µg/mL), and 10/12 isolates SXT-susceptible by Sensititre® (MIC ≤0.5 - 2 µg/mL)).

Thus, we have identified a community-associated, trimethoprim- and methicillin-resistant PVL-positive ST5 *S. aureus* clone circulating in the Northern Territory of Australia. Isolates of this ST5 clone were collected from 2011-2013 from remote communities in the Top End (coastal and inland tropical savannah) and Central Australia (arid hot desert), and urban Darwin (coastal tropical) spanning a distance of

~1,700km, indicating that this clone has successfully established itself. The emergence of a PVL-positive, trimethoprim- and methicillin-resistant ST5 *S. aureus* clone was noted in the Kimberley region of Western Australia in 2010, and now accounts for 9% of MRSA infections in that region [5]. Given the phenotypic similarities, these ST5 clones are likely related, but this has yet to be investigated.

The observed discrepancy in antimicrobial susceptibility testing results reported here is concerning. Misleading results that over-call resistance may lead to unnecessary restriction of effective oral treatment options for patients with *S. aureus* infections. In remote Indigenous communities in northern Australia where there is a high burden of staphylococcal infections and associated use of antibiotics, conditions are likely to be conducive to selection for clones with resistance to β-lactams and other oral antimicrobial agents. There has been clear evidence of the expansion of a ST5 trimethoprim-resistant clone in the neighbouring Kimberley region [5]. Whether SXT should be used to treat skin sores in remote Australian communities is contentious, with the principle question being whether the use of SXT will select SXT resistance. Accordingly, overcalling of SXT resistance has great potential to lead to non-optimal guidelines and practices.

Our findings are critical in demonstrating that the Northern Territory ST5 clone is not SXT-resistant. The drivers of this clonal expansion are still to be determined, but are unlikely to be related solely to the use of SXT. β-lactam use may be critical given the co-location of *mecA* and *dfrG*. Ongoing surveillance of antimicrobial resistance among *S. aureus* together with a more detailed understanding of the clinical significance of trimethoprim resistance are now required.

TRANSPARENCY DECLARATION

The authors have no conflicts of interest.

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Table 1 Skin Sore Trial isolates

	MIC (µg/mL)						Lab 2 MIC (µg/mL)			
	Vitek®2	Etest®					Vitek®2	Etest®		BMD[e]
Isolate[a]	**SXT[b]**	**TMP[c]**	**SXT[d]**	**ST**	**SCC*mec***	***dfrG***	**SXT**	**TMP**	**SXT**	**SXT**
SST1502_S1A_SA1	320 (16/304)	≥32	0.25	5	IVo	+				
SST1502_S2A_SA1	320 (16/304)	≥32	0.25	5	IVo	+				
SST1502_S3A_SA1	320 (16/304)	≥32	0.25	5	IVo	+	160 (8/152)	>32	0.125	2
SST1505_NS2_SA1	320 (16/304)	≥32	0.25	5	IVo	+				
SST2254_S1B_SA1	320 (16/304)	≥32	0.25	5	IVo	+				
SST2272_S2B_SA1	320 (16/304)	≥32	0.25	5	IVo	+				
SST2272_S3B_SA1	320 (16/304)	≥32	0.25	5	IVo	+				
SST2281_S1B_SA1	320 (16/304)	≥32	0.25	5	IVo	+				

SST2281_S2B_SA1	320 (16/304)	≥32	0.25	5	IVo	+				
SST2287_S1A_SA1	320 (16/304)	≥32	0.25	5	IVo	+				
SST2287_S1B_SA1	320 (16/304)	≥32	0.25	5	IVo	+				
SST2287_S2A_SA1	320 (16/304)	≥32	0.25	5	IVo	+				
SST2287_S2B_SA1	320 (16/304)	≥32	0.25	5	IVo	+				
SST2352_S1B_SA1	320 (16/304)	≥32	0.25	5	IVo	+				
SST2352_S1B_SA2	320 (16/304)	≥32	0.25	5	IVo	+				
SST2352_S2A_SA1	320 (16/304)	≥32	0.25	5	IVo	+				
SST2352_NS2_SA1	320 (16/304)	≥32	0.25	5	IVo	+				
SST2352_NS2_SA2	320 (16/304)	≥32	0.25	5	IVo	+	320 (16/304)	>32	0.125	1
SST2352_NS2_SA3	320 (16/304)	≥32	0.25	5	IVo	+				

[a] The first four numbers following 'SST' denote the study ID of the Skin Sore Trial participant from which the isolate was derived.

[b] Vitek®2 trimethoprim-sulfamethoxazole (SXT) MICs are reported as the sum of the trimethoprim and sulfamethoxazole MICs which are present in a ratio of 1:19. The SXT resistance breakpoint in this system is ≥ 80 mg/L (CLSI).

[c] TMP, trimethoprim. The EUCAST trimethoprim resistance breakpoint is >4 µg/mL.

[d] Etest® SXT MICs are reported as the trimethoprim MIC in µg/mL. The EUCAST SXT resistance breakpoint is >4 µg/mL.

[e] Broth microdilution (BMD) SXT MICs are reported as the trimethoprim MIC in µg/mL. The resistance breakpoint for this system is ≥4 µg/mL.