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Molecular Epidemiology of Staphylococcus aureus in African Children from Rural and Urban Communities with Atopic Dermatitis

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Abstract

Background: *Staphylococcus aureus* has been associated with the exacerbation and severity of atopic dermatitis (AD). Studies have not investigated the colonisation dynamics of *S. aureus* lineages in African children with AD. We determined the prevalence and population structure of *S. aureus* in children with and without AD from rural and urban South African settings.

Methods: We conducted a study of AD-affected and non-atopic AmaXhosa children from rural Umtata and urban Cape Town, South Africa. *S. aureus* was screened from skin and nasal specimens using microbiological culture, and *S. aureus* clonal lineages were determined by *spa* typing. Logistic regression analyses were employed to assess risk factors associated with *S. aureus* colonisation.

Results: *S. aureus* colonisation was higher in cases compared to controls (60% vs. 21%, p=0.000) and when stratified for location (54% vs. 13%, p=0.000 and 70% vs. 35%, p=0.005 in Umtata and Cape Town, respectively). Severe AD was associated with higher colonisation compared with moderate AD (86% vs. 52%, p=0.022) among urban cases. Having AD was associated with colonisation in both rural (odds ratio [OR] 7.54, 95% CI 2.92–19.47) and urban (OR 4.2, 95% CI 1.57–11.2) children. In rural children, living in an electrified house that also uses gas (OR 4.08, 95% CI 1.59–10.44) or in a house that uses kerosene and paraffin (OR 2.88, 95% CI 1.22–6.77) for heating and cooking were associated with increased *S. aureus* colonisation, while exposure to animals (OR 0.3, 95% CI 0.11–0.83) as well living in a house that uses wood and coal (OR 0.14, 95% CI 0.04–0.49) and an outdoor fire (OR 0.31, 95% CI 0.13–0.73) were protective. Among urban children, t272 and t1476 dominant among cases but no single *spa* type dominated among controls. In urban cases, *spa* types t002 and t442 isolates were only identified in severe AD and t174 was more frequent in moderate AD while t1476 in severe AD.

Conclusion: The strain genotype of *S. aureus* differed by AD phenotypes and rural-urban living. Continued surveillance of colonising *S. aureus* lineages is key in understanding alterations in skin microbial composition associated with AD pathogenesis and exacerbation.

Introduction

Atopic dermatitis (AD) is a common childhood inflammatory skin disease that frequently presents in early childhood.[1,2] The prevalence of AD is high in developed countries where it affects 10–20% of children.[3,4] However, recent epidemiological data indicate an increase in the prevalence of AD among children in developing countries, including South Africa.[5–7] Patients with AD usually suffer from persistent or relapsing itchy and dry eczematous skin lesions with inflammation and increased susceptibility to cutaneous *Staphylococcus aureus* colonisation.[8,9] In addition to skin colonisation, *S. aureus* has also been reported to colonise the nasal cavity where it is thought to serve as a primary reservoir for extra-nasal auto-transmission.[10,11] Skin and nasal *S. aureus* colonisation have been demonstrated in both AD patients and healthy individuals, however, with a higher colonisation density and prevalence in AD patients.[10] *S. aureus* colonisation has also been associated with AD pathogenesis,[10,12–14] with colonisation preceding the clinical onset of AD in early childhood.[15,16] Moreover, molecular epidemiological studies have shown that while colonisation occurs in both AD patients and healthy individuals, the genetic background of *S. aureus* differs

across AD disease phenotypes and may influence disease pathogenesis and severity.[2,17] The increasing AD and allergy prevalence is associated with urbanisation with a lower prevalence and microbial-related protective environmental factors noted in rural areas. The centrality of *S. aureus* in AD pathogenesis is undisputed. Whilst there are rural-urban differences in AD prevalence, the disease does occur in both settings and we sought to study the prevalence and genotypes of *S. aureus* from skin and nasal samples of South African AmaXhosa children with AD and healthy counterparts. Our secondary objectives were to evaluate the risk factors for *S. aureus* colonisation.

Materials And Methods

Study design, setting and population

Participant recruitment

We conducted a cross-sectional study of 220 children with and without AD aged 12–36 months from February 2015 to May 2016 (Fig. 1).[18,19] Urban control subjects (n = 50) were recruited as a sub-study from non-allergic, non-food-sensitised subjects participating in the South African Food Allergy (SAFFA) study at randomly selected creches in the Cape Town metropole. As creches are rarely found in the rural district, rural controls (n = 54) were recruited from children of eligible age from the areas surrounding 10 district community health clinics in the rural Mqanduli district of Umtata. Patients with moderate to severe AD (n = 56) were recruited from the Department of Paediatric Dermatology of the Red Cross War Memorial Children's Hospital in Cape Town and rural cases (n = 60) from the Department of Dermatology, Nelson Mandela Academic Hospital, in Umtata. AD was clinically diagnosed by a dermatologist using the validated UK Working Party diagnosis criteria for AD.[20] Disease severity was determined using the objective SCORAD (SCORing of Atopic Dermatitis) index into moderate (15–40) and severe (> 40).[21] Additional questionnaires gathered rich environmental exposure data in addition to the clinical data.

Specimen collection and processing

Sterile Copan nylon-tipped flocked swabs (Cat. no. 516C; Copan Italia, Brescia, Italy) were used to collect samples from lesional (i.e., most active area of eczematous skin with acute and/or chronic changes) and non-lesional skin (i.e., area with the most normal appearing skin – usually the back in young children). The swab was pre-moistened with sterile distilled water and a 4 cm^2 area of the skin lesion was swabbed for at least 1 minute in a non-overlapping manner. In addition, nasal swabs were collected from all participants to determine the *S. aureus* carriage status according to previously described methodology.[22] The collected swabs were immediately placed into 1 ml skim milk-tryptone-glucose-glycerol (STGG), transported at 4 °C to the laboratory within two hours of collection and frozen at -80 °C for subsequent batch processing. All lesional, non-lesional, and nasal swabs stored in STGG were allowed to thaw at room temperature, vortexed for 30 seconds and 100 µl was inoculated onto Mannitol Salt Agar (MSA) (National Health Laboratory Services [NHLS], Green Point Media Laboratory Cape Town, South Africa), and aerobically incubated at 37 °C for 48 hours. Isolates that were positive for both mannitol fermentation and DNase production were identified as *S. aureus*.[23] A total of 35 participants were missing specimens and were excluded from the study analysis (Fig. 1). Further, 11

participants (eight cases and three controls) had one missing specimen for either lesional skin, non-lesional skin or the anterior nares.

Nucleic acid extraction

Recovered *S. aureus* isolates were aerobically subcultured onto 2% sheep blood agar overnight. Genomic DNA extraction was completed using a modified heat lysis method.[24] Briefly, AVE buffer (Qiagen, Hilden, Germany) was used to suspend colonies instead of phosphate-buffered saline, and centrifuged at 13,000 g for two minutes. The supernatant containing genomic DNA was diluted in AVE buffer depending on the initial DNA concentration to a final concentration range of 20–70 ng/µl.

Molecular characterisation: S. aureus isolates were characterised by *spa*-typing targeting the variable X-region of the *spa* gene using the conventional primers *spa*-1113F/*spa*-1514R.[25,26] Isolates that failed to yield a *spa* amplicon or had poor sequence quality were re-amplified using the primers *spa*-T3F/*spa*-1517R or *spa*-1095F/*spa*-1517R.[27,28] Clustering was based on their genetic relatedness to *spa*-clonal complexes (*spa*-CCs) using the Based Upon Repeat Pattern (BURP) clustering algorithm of the Ridom Staph Type software (Ridom GmbH, Münster, Germany).[29] Amplification of the *nuc* gene was performed to rule out misidentification of isolates that failed to yield a *spa* amplicon. [30]

Statistical analysis

All data analyses were performed using Stata version SE16.0 (1985–2019 StataCorp LP, Texas, USA). The significance threshold for all analyses was 0.05. Univariate and multivariate analyses to assess risk factors for *S. aureus* colonisation were performed using logistic regression and presented as odd ratios (OR) reported with a 95% confidence interval (CI). The level of statistical significance in the logistic regression analysis was determined by a Chi-square test. Variables that were significant determinants of colonisation were included in the multivariate logistic regression model. Comparison of categorical data was performed by Fisher's exact test unless stated otherwise. Comparison of means was performed using the t-test for two independent samples and reported with a standard deviation (SD). Participants with missing data were excluded from the analyses relating to that variable.

Results

Characteristics of the study participants

Table 1 shows the characteristics of the case and control participants stratified by location. Of the 220 children recruited for the study (Fig. 1), 185 (111 Umtata and 74 Cape Town) were considered for analysis. The average ages of children from Umtata and Cape Town were 21.27 months old (SD ± 7.51) and 24.19 months old (SD ± 7.37), respectively. Also, the proportions of cases and controls were similar in Umtata, however, there were more cases than controls in Cape Town. Among participants from Umtata, food allergy was more common in cases compared to controls (17% [10/58] vs. 2% [1/53], p = 0.009) but was only reported in cases (21% [9/43]) in children from Cape Town. In contrast, animal exposure of the child, a history of breastfeeding and maternal animal exposure were more common among controls compared to cases in Umtata (100% [53/53] vs. 67% [39/58], p = 0.000; 47% [25/53] vs. 17% [10/58], p = 0.001; and 98% [52/53] vs. 76% [44/58], p = 0.001,

respectively). Animal exposure was only detected in 5% (2/43) of cases in Cape Town participants. Concerning sources of household heating and cooking, living in an electrified house that also uses gas (97% [56/58] vs. 25% [13/53], $p = 0.000$) and in a house that uses kerosene and paraffin (76% [44/58] vs. 38% [20/53], $p = 0.000$) were more common among cases, while living in a house that uses paraffin (60% [32/53] vs. 10% [6/58], $p = 0.000$), an outdoor fire (70% [37/53] vs. 21% [12/58], $p = 0.000$), and wood and coal (51% [27/53] vs. 7% [4/58], $p = 0.000$) were more common among controls in Umtata. In contrast, there was an overall low prevalence of children living in houses that used electricity-independent sources for heating and cooking in Cape Town.

Table 1

Participant characteristics of atopic dermatitis cases and healthy controls. Bold text indicates statistical significance. AD, atopic dermatitis; CI, confidence interval; IQR, interquartile range; [1] Large family is arbitrarily defined as 7 or more members living within one household.

Explanatory variable	Umtata				Cape Town			
	Total, n (%)	**Case, n (%)**	**Control, n (%)**	**p-value**	**Total, n (%)**	**Case, n (%)**	**Control, n (%)**	**p-value**
Total	111 (100)	58 (52)	53 (48)	0.502[a]	74 (100)	43 (58)	31 (42)	0.049[a]
Age (months)								
Mean [standard deviation]	21.27 [7.15]	21.03 [7.41]	21.53 [6.90]	0.718	24.19 [7.37]	23.98 [7.44]	24.48 [7.38]	0.773
Sex								
Female	42 (39)	24 (43)	18 (34)	0.431	36 (49)	19 (44)	17 (55)	0.480
Male	67 (61)	32 (57)	35 (66)		38 (51)	24 (56)	14 (45)	
AD severity								
Moderate	23 (40)	23 (40)	—		21 (49)	21 (49)	—	
Severe	35 (60)	35 (60)	—		22 (51)	22 (51)	—	
Atopic disease								
Food allergy	11 (10)	10 (17)	1 (2)	**0.009**	9 (12)	9 (21)	0 (0)	**0.008**
Asthma	0 (0)	0 (0)	0 (0)		1 (1)	1 (3)	0 (0)	1.000
Allergic rhinitis	7 (8)	1 (2)	6 (11)	0.242	1 (1)	1 (3)	0 (0)	1.000
Mode of birth								
Caesarean section	25 (23)	14 (24)	11 (21)	0.821	33 (46)	20 (49)	13 (42)	0.637
Vaginal	86 (77)	44 (76)	42 (79)		39 (54)	21 (51)	18 (58)	
Breastfeeding	35 (32)	10 (17)	25 (47)	**0.001**	9 (12)	7 (16)	2 (6)	0.288
Antibiotic exposure	92 (82)	49 (83)	43 (81)	0.810	54 (72)	30 (70)	24 (77)	0.598
Immunisation status								
Complete	107 (96)	56 (95)	52 (98)	0.620	64 (86)	33 (77)	31 (100)	0.004
Incomplete	4 (4)	3 (5)	1 (2)		10 (14)	10 (23)	0 (0)	
Large family [1]	62 (55)	30 (52)	32 (60)	0.445	24 (32)	14 (33)	10 (32)	1.000
Animal exposure	93 (84)	39 (67)	53 (100)	**0.000**	2 (3)	2 (6)	0 (0)	0.495

Explanatory variable	Umtata				Cape Town			
	Total, n (%)	Case, n (%)	Control, n (%)	p-value	Total, n (%)	Case, n (%)	Control, n (%)	p-value
Parental education								
Primary	8 (7)	2 (3)	6 (11)	**0.000**	1 (1)	1 (2)	0 (0)	**0.025**
Secondary	70 (63)	31 (53)	39 (74)		33 (45)	14 (33)	19 (61)	
Tertiary	31 (28)	25 (43)	6 (11)		40 (54)	28 (65)	12 (39)	
Other	2 (2)	0 (0)	2 (4)		0 (0)	0 (0)	0 (0)	
Maternal factors								
Animal exposure	96 (86)	44 (76)	52 (98)	**0.001**	4 (60)	4 (11)	0 (0)	0.120
Pregnant smoking	1 (1)	0 (0)	1 (2)	0.482	3 (45)	0 (0)	3 (10)	0.094
Smoking	1 (1)	0 (0)	1 (2)	0.477	4 (6)	1 (3)	3 (10)	0.324
Asthma	2 (2)	2 (3)	0 (0)	0.496	6 (8)	4 (10)	2 (6)	1.000
Allergic rhinitis	4 (4)	4 (7)	0 (0)	0.120	5 (68)	4 (10)	1 (3)	0.387
Atopic dermatitis	2 (2)	2 (3)	0 (0)	0.496	3 (4)	2 (5)	1 (3)	1.000
Food allergy	3 (3)	2 (3)	1 (2)	1.000	1 (1)	1 (2)	0 (0)	1.000
Paternal factors								
Smoking	15 (14)	9 (16)	6 (12)	0.589	20 (31)	11 (31)	9 (31)	1.000
Asthma	3 (3)	3 (5)	0 (0)	0.245	0 (0)	0 (0)	0 (0)	
Allergic rhinitis	3 (3)	3 (5)	0 (0)	0.245	7 (10)	7 (17)	0 (0)	0.018
Atopic dermatitis	1 (1)	1 (2)	0 (0)	1.000	2 (3)	2 (5)	0 (0)	0.505
Food allergy	1 (1)	1 (2)	0 (0)	1.000	1 (1)	1 (2)	0 (0)	1.000
Household factors								
Electricity + gas	69 (62)	56 (97)	13 (25)	**0.000**	66 (99)	35 (97)	31 (100)	1.000
Kerosene + paraffin	64 (58)	44 (76)	20 (38)	**0.000**	43 (64)	21 (58)	22 (71)	0.317
Paraffin	38 (34)	6 (10)	32 (60)	**0.000**	0 (0)	0 (0)	0 (0)	
Indoor fire	4 (4)	2 (3)	2 (4)	1.000	0 (0)	0 (0)	0 (0)	
Outdoor fire	49 (44)	12 (21)	37 (70)	**0.000**	0 (0)	0 (0)	0 (0)	
Wood + fire	31 (28)	4 (7)	27 (51)	**0.000**	0 (0)	0 (0)	0 (0)	

Distribution of skin and nasal *S. aureus* colonisation

A total of 185 (84 controls and 101 cases) children were assessed for *S. aureus* colonisation. Of these, 79 (43%) were colonised with *S. aureus* in at least one of the sampled body sites. Amongst all the study children, *S. aureus* was recovered more frequently from cases compared to controls (60% [61/101] vs. 21% [18/84], p = 0.000). Participants from urban settings were frequently colonised compared to rural participants (55% [41/74] vs. 34% [38/111], p = 0.006). Stratification by location revealed that in both rural and urban settings, *S. aureus* was more commonly detected from cases compared to controls (54% [31/58] vs. 13% [7/53], p = 0.000 and 70% [30/43] vs. 35% [11/31], p = 0.005), respectively. Data on the prevalence of colonisation by sampled body site in cases and controls from both locations are summarised in Table 2. Among all children and rural children, cases were more frequently colonised on non-lesional skin compared to controls (33% [32/97] vs. 12% [10/83], p = 0.001, and 29% [16/55] vs. 10% [5/52], p = 0.015, respectively), but there was no significant difference between urban cases and controls (Table 2). Further, the anterior nares were commonly colonised in cases compared to controls among all children (28% [28/99] vs. 15% [12/82], p = 0.031), but not when stratified by location. Comparative analysis of the prevalence of colonisation on sampled site by disease phenotype revealed that there was a higher rate of detecting *S. aureus* on lesional skin compared to the anterior nares among all cases (p = 0.003) and rural cases (p = 0.02), but not in urban cases (p = 0.212). Colonisation of lesional skin was more common than on non-lesional skin in all cases (p = 0.002) and urban cases (p = 0.029), but not rural cases (p = 0.06). Further, we noted significant difference in the colonisation of non-lesional skin and the anterior nares across all controls (p = 0.037) and rural controls (p = 0.005), but not among urban controls (p = 1.000).

Table 2
Participant colonisation among all, rural and urban cases and controls.

	Total			**Umtata**			**Cape Town**		
	Case, *n* (%)	**Control, *n* (%)**	**p-value**	**Case, *n* (%)**	**Control, *n* (%)**	**p-value**	**Case, *n* (%)**	**Control, *n* (%)**	**p-value**
Lesional skin	42 (42)			22 (39)			20 (48)		
Non-lesional skin	32 (33)	10 (12)	0.001	16 (29)	5 (10)	0.015	16 (38)	5 (16)	0.066
Anterior nares	28 (28)	12 (15)	0.031	9 (16)	5 (10)	0.396	19 (44)	7 (23)	0.085

S. aureus **colonisation and AD severity**

Overall, cases with severe disease were more commonly colonised compared to cases with moderate disease (72% [41/57] vs. 47% [20/43], p = 0.013). Stratification for location revealed that only urban cases with severe disease were frequently colonised compared to moderate AD cases (86% [19/22] vs. 52% [11/21], p = 0.022). More specifically, colonisation on lesional skin, non-lesional skin and the anterior nares was not significantly associated with disease severity among all cases as well as when stratified by location (Table 3).

Table 3
Colonisation in cases stratified by disease severity among all, rural and urban cases.

	Total			**Umtata**			**Cape Town**		
	Moderate, *n* (%)	**Severe, *n* (%)**	**p-value**	**Moderate, *n* (%)**	**Severe, *n* (%)**	**p-value**	**Moderate, *n* (%)**	**Severe, *n* (%)**	**p-value**
Lesional skin	13 (32)	29 (51)	0.066	5 (24)	17 (49)	0.092	8 (40)	12 (55)	0.374
Non-lesional skin	13 (30)	19 (36)	0.665	6 (27)	10 (31)	1.000	7 (33)	9 (43)	0.751
Anterior nares	11 (26)	17 (31)	0.655	3 (14)	6 (18)	0.727	8 (38)	11(50)	0.543

Risk factors associated with S. aureus **colonisation across the locations**

Geographic location influenced microbial colonisation on the skin and nasal cavity. Namely, the effect of various risk factors on being colonised with *S. aureus* in children from both locations using logistic regression are shown in Tables 4a and 4b, for rural and urban children, respectively. The univariate analysis models showed that having AD was associated with colonisation in both rural (OR 7.54, 95% CI 22.92–19.47) and urban (OR 4.2, 95% CI 1.57–11.2) children. Also, living in an electrified house that also uses gas (OR 4.08, 95% CI 1.59–10.44) and in a house that makes use of kerosene and paraffin (OR 2.88, 95% CI 1.22–6.77) for heating and cooking were associated with an increased risk of *S. aureus* among the rural children. Surprisingly, exposure to animals (OR 0.3, 95% CI 0.11–0.83) as well living in a house that uses wood and coal (OR 0.14, 95% CI 0.04–0.49) and an outdoor fire (OR 0.31, 95% CI 0.13–0.73) were associated with lower odds of colonisation. Nonetheless, in the multivariate model of rural children, having AD (aOR 8.02, 95% CI 1.28–50.37) was retained as a risk factor for *S. aureus* colonisation, while living in a house that uses wood and coal for cooking and heating (aOR 0.02, 95% CI 0.02–0.99) remained protective against *S. aureus* colonisation. No regression analysis was performed for urban children because only AD showed an association with *S. aureus*.

Table 4

a. Unconditional logistic regression analysis of child, parental, domestic and environmental characteristics associated with *S. aureus* colonisation in Umtata participants.

Explanatory variable	**Colonised [a], *n* (%)**	**Not colonised, *n* (%)**	**OR [95% CI]**	**p-value**	**aOR [95% CI]**	**p-value**
AD: case	31 (28)	27 (24)	7.54 [2.92–19.47]	**0.000**	8.02 [1.28–50.37]	**0.026**
Sex: male	21 (19)	46 (42)	0.74 [0.33–1.67]	0.469	0.83 [0.32–2.16]	0.696
Child characteristics						
Breastfeeding	10 (9)	25 (23)	0.69 [0.29–1.63]	0.395	1.46 [0.48–4.47]	0.503
Allergic rhinitis	1 (1)	6 (7)	0.43 [0.05–3.79]	0.449	Excluded	
Asthma [§]	0 (0)	0 (0)	Omitted [d]		Excluded	
Food allergy	5 (5)	6 (5)	1.69 [0.48–5.95]	0.413	Excluded	
Mode of delivery: vaginal	29 (26)	57 (51)	0.9 [0.36–2.29]	0.833	Excluded	
Incomplete immunisation status	2 (2)	2 (2)	1.97 [0.27–14.58]	0.506	Excluded	
Antibiotic exposure	33 (30)	58 (52)	1.71 [0.57–5.12]	0.34	1.54 [0.39–6]	0.536
Large family size [b]	15 (14)	35 (32)	0.71 [0.32–1.57]	0.395	0.94 [0.36–2.44]	0.903
Animal exposure [c]	27 (24)	65 (59)	0.3 [0.11–0.83]	**0.021**	0.53 [0.11–2.54]	0.429
Fossil fuel exposure						
Electricity + gas	31 (28)	38 (34)	4.08 [1.59–10.44]	**0.003**	0.35 [0.05–2.47]	0.295
Kerosene + paraffin	28 (25)	36 (32)	2.88 [1.22–6.77]	**0.015**	0.69 [0.19–2.49]	0.571
Indoor fire	1 (1)	3 (3)	0.63 [0.06–6.27]	0.694	Excluded	
Outdoor fire	10 (9)	39 (35)	0.31 [0.13–0.73]	**0.008**	0.54 [0.17–1.67]	0.283

AD, atopic dermatitis; OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval; [§] No within group variance; [a] Colonisation with *Staphylococcus aureus*; [b] Large family size is arbitrarily defined as 7 or more members within a household; [c] Animal exposure refers to cat and/or dog exposure (exposure to a domestic animal); [d] Independent variables omitted due to dependency in the regression model

Explanatory variable	Colonised [a], *n* (%)	Not colonised, *n* (%)	OR [95% CI]	p-value	aOR [95% CI]	p-value
Wood + coal	3 (3)	28 (25)	0.14 [0.04–0.49]	**0.002**	0.14 [0.02–0.99]	**0.048**
Maternal factors						
Allergic rhinitis	0 (0)	4 (4)	Omitted [d]		Excluded	
Asthma	1 (1)	1 (1)	1.95 [0.12–32]	0.641	Excluded	
Atopic dermatitis	1 (1)	1 (1)	1.95 [0.12–32]	0.641	Excluded	
Food allergy	1 (1)	2 (2)	0.96 [0.08–10.93]	0.973	Excluded	
Smoking	0 (0)	1 (1)	Omitted		Excluded	
Pregnant smoker	0 (0)	1 (1)	Omitted		Excluded	
Animal exposure [c]	31 (28)	65 (59)	0.55 [0.18–1.64]	0.28	1.93 [0.37–10.16]	0.438
Paternal factors						
Allergic rhinitis §	0 (0)	3 (3)	Omitted		Excluded	
Asthma §	1 (1)	2 (2)	0.96 [0.08–10.93]	0.973	Excluded	
Atopic dermatitis §	0 (0)	1 (1)	Omitted		Excluded	
Food allergy §	0 (0)	1 (1)	Omitted		Excluded	
Smoking	5 (5)	10 (9)	0.94 [0.3–2.98]	0.267	Excluded	

AD, atopic dermatitis; OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval; § No within group variance; [a] Colonisation with *Staphylococcus aureus*; [b] Large family size is arbitrarily defined as 7 or more members within a household; [c] Animal exposure refers to cat and/or dog exposure (exposure to a domestic animal); [d] Independent variables omitted due to dependency in the regression model

Table 4
b. Unconditional logistic regression analysis of child, parental, domestic and environmental characteristics associated with *S. aureus* colonisation in Cape Town participants.

Explanatory variable	**Colonised [a], *n* (%)**	**Not colonised, *n* (%)**	**OR [95% CI]**	**p-value**
AD: case	30 (41)	13 (18)	4.2 [1.57–11.2]	**0.004**
Sex: male	19 (26)	19 (25)	0.74 [0.33–1.67]	0.469
Child characteristics				
Breastfeeding	6 (8)	3 (4)	1.71 [0.39–7.45]	0.472
Atopic dermatitis				
Allergic rhinitis	1 (1)	0 (0)	Omitted [d]	
Asthma [§]	1 (1)	0 (0)	Omitted [d]	
Food allergy	6 (8)	3 (4)	1.71 [0.39–7.45]	0.472
Mode of delivery: vaginal	22 (31)	17 (24)	0.95 [0.37–2.43]	0.921
Incomplete immunisation status	8 (11)	2 (3)	3.76 [0.74–19.09]	0.11
Antibiotic exposure	31 (42)	23 (31)	1.35 [0.48–3.77]	0.57
Large family size [b]	9 (12)	8 (11)	0.88 [0.3–2.61]	0.816
Animal exposure [c]	1 (1)	1 (1)	0.86 [0.05–14.3]	0.915
Fossil fuel exposure				
Electricity + gas	36 (54)	30 (45)	Omitted [d]	
Kerosene + paraffin	20 (30)	23 (34)	0.43 [0.15–1.23]	0.116
Indoor fire	0 (0)	0 (0)	Omitted [d]	
Outdoor fire	0 (0)	0 (0)	Omitted [d]	
Wood + coal	0 (0)	0 (0)	Omitted [d]	
Maternal factors				
Allergic rhinitis	0 (0)	5 (7)	Omitted [d]	
Asthma	3 (4)	3 (4)	0.76 [0.14–4.06]	0.751

AD, atopic dermatitis; OR, odds ratio; CI, confidence interval; [§] No within group variance; [a] Colonisation with *Staphylococcus aureus*. [b] Large family size is arbitrarily defined by more than 6 members within a household; [c] Animal exposure refers to cat and/or dog exposure (exposure to a domestic animal); [d] Independent variables omitted due to dependency in the regression model.

Explanatory variable	Colonised [a], *n* (%)	Not colonised, *n* (%)	OR [95% CI]	p-value
Atopic dermatitis	1 (1)	2 (3)	0.38 [0.03–4.33]	0.432
Food allergy	1/73	0 (0)	Omitted [d]	
Smoking	3 (4)	1 (1)	2.65 [0.26–26.82]	0.41
Pregnant smoker	2 (3)	1 (1)	1.76 [0.15–20.45]	0.65
Animal exposure [c]	3 (5)	1 (2)	2.64 [0.26–26.76]	0.412
Paternal factors				
Allergic rhinitis [§]	5 (7)	2 (3)	2.08 [0.38–11.52]	0.4
Asthma [§]	0 (0)	0 (0)	Omitted [d]	
Atopic dermatitis [§]	2 (3)	0 (0)	Omitted [d]	
Food allergy [§]	1 (1)	0 (0)	Omitted [d]	
Smoking	13 (20)	7 (11)	1.86 [0.62–5.54]	0.267

AD, atopic dermatitis; OR, odds ratio; CI, confidence interval; [§] No within group variance; [a] Colonisation with *Staphylococcus aureus*. [b] Large family size is arbitrarily defined by more than 6 members within a household; [c] Animal exposure refers to cat and/or dog exposure (exposure to a domestic animal); [d] Independent variables omitted due to dependency in the regression model.

Clonal lineages of recovered S. aureus isolates

A total of 125 skin and nasal *S. aureus* isolates were recovered from cases and controls, however, only 108 isolates were characterised by *spa* typing (Fig. 1). Seventeen isolates were excluded from molecular analysis due to their failure to amplify the *spa* gene using the described primers or poor sequence quality for *spa* type assignment despite repeated sequencing. BURP analysis grouped 19 *spa* types into 6 *spa*-clonal complexes (*spa*-CCs) and 15 *spa* types were singletons. Among participants with *spa* typed isolates, 25% (19/76) were colonised with one *spa* type on all three or two of the sampled sites which were positive for *S. aureus*, while 7% (5/76) were colonised with different *spa* types. One rural case participant was colonised with *spa* type t062 on lesional skin and anterior nares, and with *spa* type t1399 on non-lesional skin which belongs to the same *spa*-CC. The most frequent *spa* types were *spa*-CC002/t002 (*spa*-CC/*spa* type; 8%), *spa* cluster 4/t272 (9%), *spa* cluster 6/t174 (14%) and *spa* cluster 5/t1476 (18%). Further, we identified five previously unassigned *spa* types (i.e., txAC, txAE, t18354, t15783 and t18750).

Distribution of S. aureus spa clonal lineages across locations by AD disease and severity

Rural and urban children were colonised by different *S. aureus spa* clonal lineages, with *spa* cluster 4 *spa* types frequently identified among rural participants (18% [9/51] vs. 4% [2/57], p = 0.015) and *spa* cluster 6 *spa* types in urban participants (23% [13/57] vs. 6% [3/51], p = 0.013) compared to their respective counterparts based on all sampled sites (Table 5). The diversity of *spa* types among cases was higher compared to controls in both locations (Fig. 2). Moreover, comparative analysis revealed that there was an overall significant difference in the distribution of *spa* clonal lineages between urban cases and controls (p = 0.009), with *spa* cluster 5/t1476 and *spa* cluster 6/t174 being predominant among cases. There was no overall difference between rural cases and controls (p = 0.224), albeit, *spa* cluster 4/t272 and *spa* cluster 5/t1476 were the dominant *spa* clonal lineages among cases with no single most dominant *spa* clonal lineage among controls (Fig. 2). We also noted a significant difference in the distribution of *spa* clonal lineages among urban cases based on AD severity (p = 0.001). In these cases, *spa*-CC002 (t002 and t442) isolates were only identified in severe AD, and *spa* cluster 6/t174 was more frequent in moderate AD while *spa* cluster 5/t1476 in severe AD. Further, although no significant difference was found between AD severity and the identified *spa* types in rural cases (p = 0.126), *spa* cluster 3 (t062 and t1399) isolates were only detected in moderate cases while *spa* cluster 5 (t1476 and t1257) isolates predominated in severe cases (Fig. 3).

Table 5
Distribution of clonal lineages of S. aureus **isolates among Umtata and Cape Town participants**.

***spa*-CC**	**Umtata**			**Cape Town**		
	No. of isolates (%)	**No. of *spa* types (%)**	***spa* types (no. of isolates)**	**No. of isolates (%)**	**No. of *spa* types (%)**	***spa* types (no. of isolates)**
spa-CC002	9	3 (14)	t002 (4); **t045 (2)**; **t071 (3)**	10	4 (19)	t002 (5); **t1215 (2)**; **t18748 (1)**; **t442 (2)**
spa-CC084	3	2 (10)	t084 (2); **t491 (1)**	5	2 (10)	t084 (3); **t346 (2)**
spa cluster 3	3	2 (10)	t062 (2); **t1399 (1)**	3	2 (10)	t062 (1); **t2049 (2)**
spa cluster 4	9	2 (10)	**t159 (1)**; t272 (8)	2	1 (5)	t272 (2)
spa cluster 5	12	2 (10)	t1476 (10); **t1257 (2)**	10	2 (10)	t1476 (9); **t18750 (1)**
spa cluster 6	3	1 (5)	t174 (3)	13	2 (10)	t174 (12); **t5471 (1)**
Singletons	10	7 (33)	t015 (2); **t148 (1)**; **t2763 (1); t317 (3);** t355 (1); **t786 (1); t843 (1)**	13	7 (33)	t015 (2); **t18354 (1); t1597 (1); t2078 (4);** t335 (2); **t881 (1); t891 (2)**
Excluded/*spa* types with unknown repeat succession	2	2 (10)	txAC (1); txAE (1)	1	1 (5)	**t15783 (1)**
Total	51	21		57	21	

Bold text indicates *spa* types that were identified in only one location.

Discussion

We conducted a cross-sectional, case-control study in South African AmaXhosa children from rural Umtata and urban Cape Town to determine the molecular epidemiology of *S. aureus* lineages colonising the skin and nasal cavity of AD-affected children compared to healthy children. We observed a higher prevalence of colonisation in cases compared to controls regardless of location. The distribution of *S. aureus spa* clonal lineages differed between rural-urban settings and were differentially associated with AD disease and severity. Further, determinants of *S. aureus* colonisation varied across the rural-urban settings.

The pathogenesis of AD is characterised by epidermal barrier defects resulting from a synergistic reduction in epidermal barrier structural proteins, alteration in lipid composition and skin pH, activation of inflammatory responses leading to impaired clearance of skin pathogens and a decrease in skin microbiome diversity.[14,31,32]

S. aureus dominance is consistently linked with acute AD flares and severe forms of the disease.[12,31] We noted an overall higher prevalence of *S. aureus* colonisation among cases compared to controls (60% vs. 21%). These findings are consistent with a similar study in Italy which found a prevalence of 57% vs. 20% in cases compared to control children.[33] The higher frequency of colonisation in cases compared to controls was also noted when stratified for location, supporting the reported relationship between *S. aureus* predominance and AD regardless of population and location. [10,33] With respect to sampling site-specific colonisation, AD-lesional skin has been shown to be more susceptible to *S. aureus* colonisation compared to AD-uninvolved, non-lesional skin, with a reported prevalence of colonisation of 23–70% vs. 6–39%.[10,34,35] Similarly, we noted higher frequency of colonisation on lesional skin compared to non-lesional skin among urban cases, but not in rural cases. Further, similar colonisation rates on lesional skin and anterior nares have been reported in AD with nasal colonisation thought to be the main source of the increased skin colonisation in AD.[10,13] However, we presently found that lesional skin was more frequently colonised compared to the anterior nares among rural cases suggesting a non-nasal source of *S. aureus* for the increased colonisation on lesional skin in rural AD.[33]

Skin barrier dysfunction in AD lesions, particularly in severe AD, has been correlated with increased *S. aureus* colonisation.[14,36] In agreement with recent studies,[10,14,35] we noted a higher prevalence of colonisation based on all sampled sites in cases with severe AD, however, this was limited to urban cases and not rural cases. Geographical location is thought to influence microbial colonisation and may explain the varied susceptibility of geographical populations to skin pathologies.[37] In this regard, the study communities each represent a geographic population that is uniquely affected by *S. aureus* colonisation in the pathophysiology of AD. Moreover, the rural and urban populations, regardless of disease, are generally different populations with distinct sensitisation patterns to environmental exposures[18] and inflammatory immune responses.[38] These may in turn affect microbial colonisation and the contribution thereof to disease pathogenesis and pathophysiology.

Risk factors for bacterial colonisation on the skin and nasal cavity differ with rural-urban living.[39,40] The association between *S. aureus* colonisation and AD is well studied, with some studies reporting colonisation to precede the onset of clinically appreciable AD in children and is further associated with disease severity.[15] Consistent with previous reports,[12] having AD in both communities was associated with *S. aureus* colonisation. Exposure to air pollutants has been associated with increased skin barrier damage[41] which increases the propensity to *S. aureus* colonisation.[42] In rural children, we found that living in a house that uses kerosene and paraffin which release fine air particulates[43] was associated with increased *S. aureus*. Exposure to the burning of wood/coal or an outdoor fire, which also release fine air pollutants that may induce cutaneous irritation, was associated with reduced *S. aureus* colonisation in rural children. The effect of environmental air pollutants in children is a function of time spent being exposed,[44] therefore, although the children are living in homes that use wood/coal or an outdoor fire for cooking and heating, they might have limited exposure to the produced particulates which restrict the possible effect on skin irritation and susceptibility to *S. aureus*. Electricity and biogas are relatively "clean fuels" with minimal air pollution emission at the household level.[45] In contrast, we found that rural living in an electrified house that also uses gas increased the risk of *S. aureus* colonisation. Further, animals are reservoirs for human *S. aureus* colonisation,[46] however, we found that rural children living in a house with animals was associated with a reduced risk of *S. aureus* colonisation. Similarly, this finding

could be due to the absence of direct interaction between children and animals hence there are no animal-to-human *S. aureus* transmission events. Nonetheless, AD remained a risk factor while living in a house that uses wood and coal was protective against *S. aureus* colonisation in rural children in the multivariate regression model. These findings highlight the importance of the immediate environment in shaping bacterial colonisation dynamics and the potential implication thereof in AD pathogenesis.

In addition to microbial colonisation, geographic location also determined the genotype of the colonising bacteria.[47] We noted heterogeneity in the distribution of the colonising *spa* clonal lineages based on geographic location, with rural children mostly colonised by *spa* types belonging to the *spa* cluster 4 (previously associated with MLST CC121, Table S1)[48] while urban children were predominantly colonised with *spa* cluster 6 (CC1) isolates.[49] This suggests that location may play a role in the colonisation dynamics of childhood skin and nares in the child populations.[2,17,50,51] Further, urban cases and controls exhibited distinct *S. aureus spa* clonal lineages, however, there was no difference in the distribution of *S. aureus* lineages between rural cases and controls. These findings suggest that the rural-urban locations provide a specific niche allowing for the selection of certain *S. aureus* clonal lineages which sequentially influence the location population structure and associated colonisation dynamics. Future studies are essential to investigate location-specific features in this cohort that contribute to the observed *S. aureus* population structures and their association with disease phenotype.

The relationship between disease severity and the clonal lineages of the colonising *S. aureus* isolates is unclear with some studies reporting an association between specific clonal lineages and AD severity[17,50] and others demonstrating none.[13,52] In spite of this, we noted different distributions of *S. aureus* clonal lineages depending on AD severity among urban cases. Here, *spa* clonal lineages *spa* cluster 5 (CC5)[53] and *spa* cluster 6 (CC1)[49] were the most common in severe and moderate AD, respectively. Further, *spa*-CC002 (CC5)[17] isolates were only detected in severe AD cases. These findings are in agreement with a study in Spanish children which reported a predominance of CC5 isolates in severe AD[50] and another which reported the predominance of CC1 isolates in moderate AD,[13] but contrast a report of the predominance of CC5 in moderate disease among Canadian children with AD.[17] Contrastingly, there was difference in the distribution of *spa* clonal lineages among rural cases based on disease severity. Albeit, *spa* cluster 3 (CC5)[48,54] isolates were only identified in rural cases with moderate AD and *spa* cluster 6 (CC1)[49] isolates were frequent in rural cases with severe AD. The predominance of *spa* cluster 3 (CC5) isolates is similar to that noted in moderate AD elsewhere[17] while that of *spa* cluster 6 (CC1) isolates in severe AD contrasts previous reports of the high prevalence of CC1 isolates in children with moderate AD.[13] The contrasting predominance of *S. aureus* clonal lineages based on disease severity across the rural-urban communities emphasises the importance of the environment in the contribution of bacterial clonality in disease. Therefore, more investigations are needed to determine if certain *S. aureus* clonal lineages are associated with differential AD disease severity and the concomitant contribution to AD and disease severity.

Our data are subject to a few limitations. BURP analyses are limited to *spa* types that pass the parameter of a certain number of repeats, which excludes *spa* types with the number of repeats below the set parameter.[29] In this study, the *spa* type t15783 was excluded from BURP clustering analyses due to possessing $\leq$ 5 repeats. We predicted the corresponding MLST sequence types (STs) and CCs of the *S. aureus spa* types identified in

this study by extrapolating data from previous studies (Table S1). Furthermore, 14% (17/125) of the isolates were untypeable which highlights the need for whole genome sequencing (WGS) to provide both *spa* and MLST data for detailed characterisation.[2]

Conclusion

In conclusion, our study shows that children with AD are more frequently colonised with *S. aureus* compared to non-AD controls. Moreover, the genetic background of colonising *S. aureus* is a unique signature of AD and disease severity, however, this is largely dependent on rural-urban living. These findings highlight the important of geographic location on the colonisation epidemiology and population structure of *S. aureus* as well as the associated colonisation determinants in childhood health and AD disease in South Africa. Future studies should look into the mechanisms within the rural-urban environments that contribute to the discrepancy in the *S. aureus* colonisation dynamics and the association thereof with AD and disease severity. This information will provide insights into population-specific therapeutic strategies that may be harnessed in the restoration of microbial diversity in AD-affected children.

Declarations

Ethics approval

The study was approved by the Human Research and Ethics Committee of the Faculty of Health Science, University of Cape Town (HREC/REF: 451/2014) and the Western Cape Provincial Child Health Research Committee. Written, informed consent was obtained from the participants' parents or guardian at recruitment. Enrolment of participants and all procedures were conducted in accordance with the relevant regulations and guidelines.

Consent of publication

Not applicable.

Availability of data and materials

Materials and data can be shared upon request through the corresponding author.

Competing interests

The authors declare no competing interests.

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Author contributions

FSD, MEL, CH, NL, MPN, SMA, REA and AOS conceptualised and supervised this study. FSD, NL, CH and MEL obtained funding. NL collected all clinical specimens. GONN, FSD, SMA and REA performed the experiments, data collection and analysis with support from DJ and AOS. GONN and FSD prepared the first draft manuscript. All authors contributed to manuscript review.

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Figures

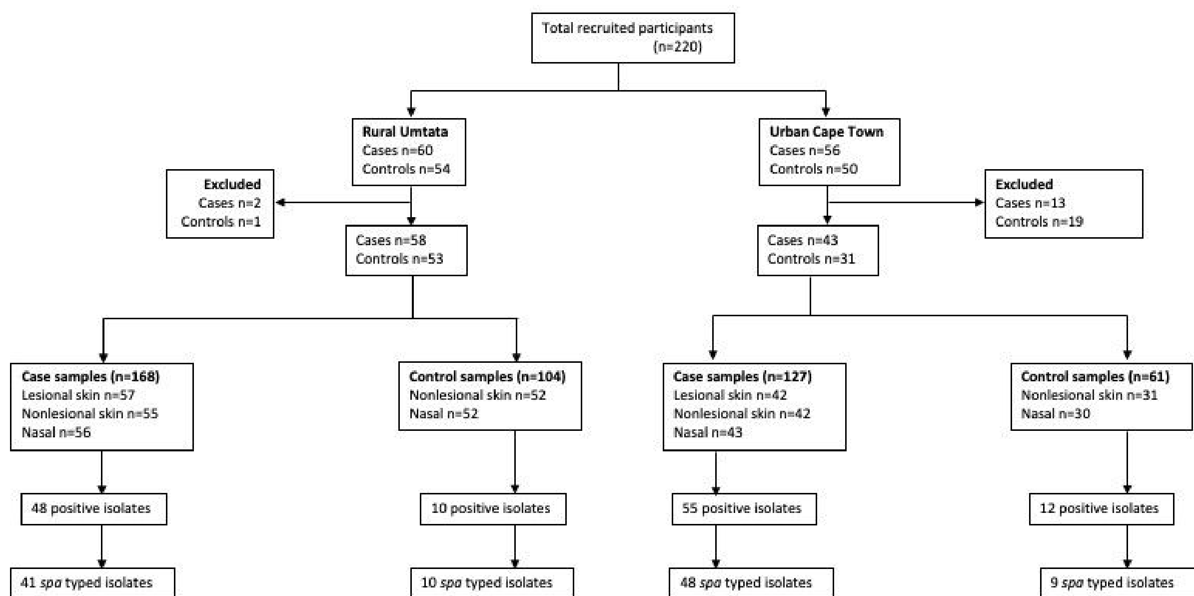

Figure 1

Flow chart of participants' sample processing.

Fig 2. case/control spa types

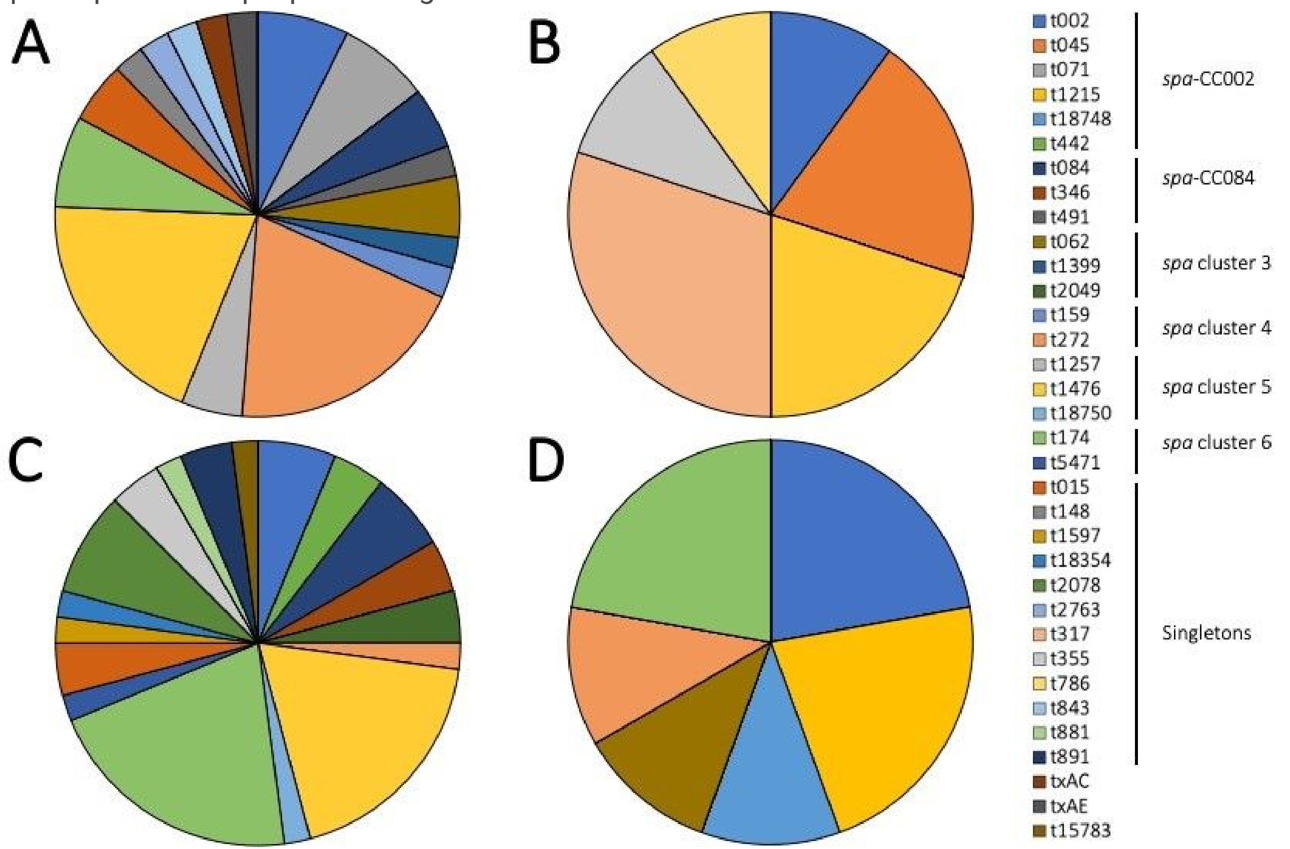

Figure 2

Distribution of spa types by disease phenotype stratified by location. (A) rural case, (B) rural control, (C) urban case, and (D) urban control. Percentages calculated by number of isolates for a spa type divided by the total number of spa types in each group.

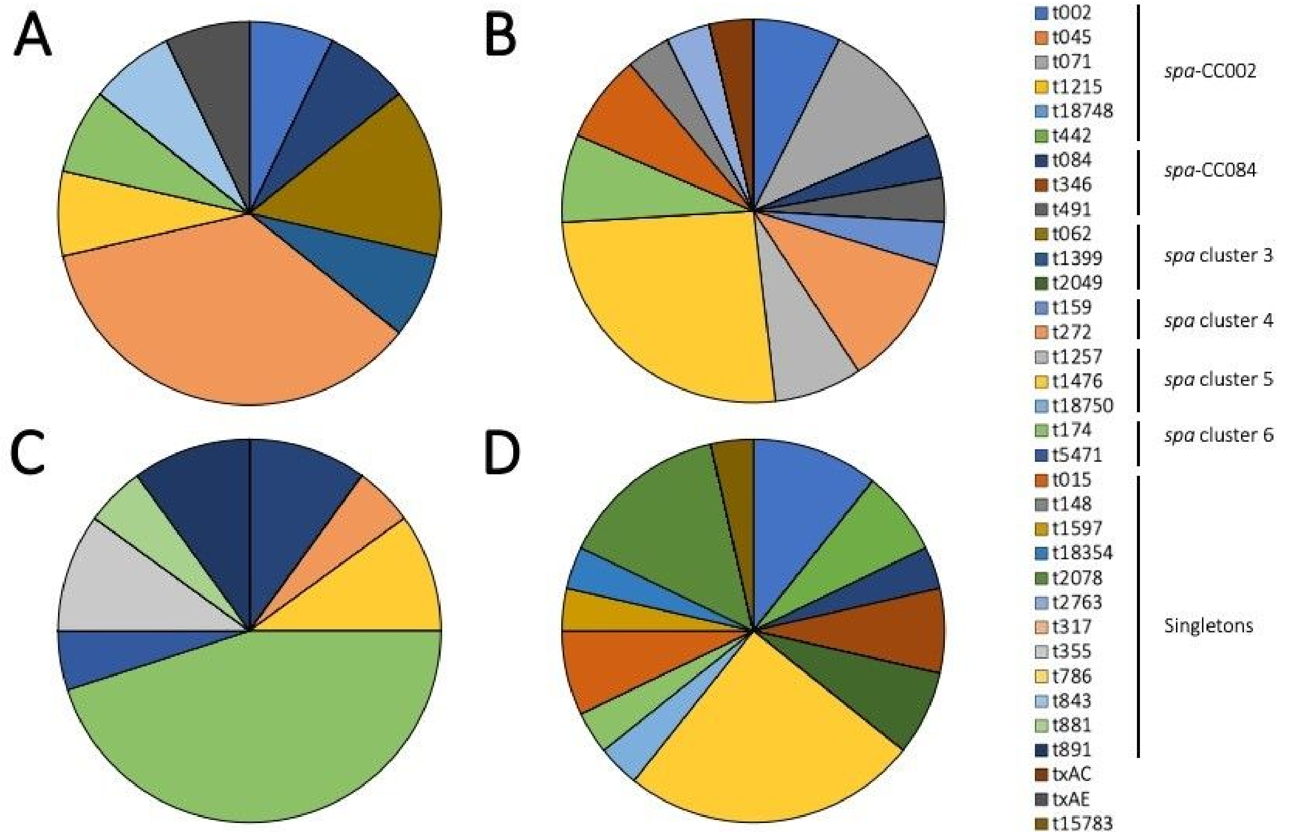

Figure 3

Distribution of spa types by disease severity. (A) rural moderate, (B) rural severe, (C) urban moderate, and (D) urban severe. Percentages calculated by number of isolates for a spa type divided by the total number of spa types in each group.

Supplementary Files

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- Supplementarydata.docx