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Original Article

spa typing and antimicrobial resistance of *Staphylococcus aureus* from healthy humans, pigs and dogs in Tanzania

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Abstract

Introduction: *Staphylococcus aureus* is an opportunistic pathogen causing infections in humans and animals. Here we report for the first time the prevalence of nasal carriage, *spa* typing and antimicrobial resistance of *S. aureus* in a Tanzanian livestock community.
Methodology: Nasal swabs were taken from 100 humans, 100 pigs and 100 dogs in Morogoro Municipal. Each swab was enriched in Mueller Hinton broth with 6.5% NaCl and subcultured on chromogenic agar for *S. aureus* detection. Presumptive *S. aureus* colonies were confirmed to the species level by *nuc* PCR and analysed by *spa* typing. Antimicrobial susceptibility patterns were determined by disc diffusion method.
Results: *S. aureus* was isolated from 22 % of humans, 4 % of pigs and 11 % of dogs. A total of 21 *spa* types were identified: 13, 7 and 1 in human, dogs, and pigs, respectively. Three *spa* types (t314, t223 and t084) were shared between humans and dogs. A novel *spa* type (t10779) was identified in an isolate recovered from a colonized human. Antimicrobials tested revealed resistance to ampicillin in all isolates, moderate resistances to other antimicrobials with tetracycline resistance being the most frequent.
Conclusion: *S. aureus* carrier frequencies in dogs and humans were within the expected range and low in pigs. The *S. aureus spa* types circulating in the community were generally not shared by different hosts and majority of types belonged to known clones. Besides ampicillin resistance, moderate levels of antimicrobial resistance were observed irrespective of the host species from which the strains were isolated.

Key words: Antimicrobials; genotyping; *S. aureus*; Tanzania.

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Introduction

Staphylococcus aureus is an opportunistic pathogen often carried asymptomatically by humans and animals. Approximately 25%-30 % of healthy humans carry *S. aureus* in their nasal cavity, and nasal carriage is a recognized risk factor for *S. aureus* infection [1,2]. Other hosts of *S. aureus* include pigs, where a similar prevalence of carriage as in humans has been reported in Switzerland [3]. *S. aureus* can also be found in dogs and cats; however, in dogs a related coagulase-positive staphylococcal specie, *S. pseudintermedius*, is more prevalent than *S. aureus* [4]. According to previous studies [5], dogs could be a possible source of *S. aureus* infection to humans, and it was demonstrated previously [6] that undistinguishable *S. aureus* strains have been isolated among human and dogs from the same geographical area.

The sequence-based typing method, *S. aureus* protein A *spa* typing, offers excellent intra- and inter-laboratory reproducibility and it is commonly applied for population studies of staphylococci [7]. A high number of types are recognized in the international database representing typing of more than 300,000 strains (http://www.spaserver.ridom.de). There are reports on *spa* types in hospital settings in Tanzania [8], but it is unknown which types circulate in the community. In addition to the general lack of information on the *S. aureus* lineages that are present in human and animal populations in Tanzania, there is also a lack of knowledge of the patterns of antimicrobial susceptibility to commonly used antimicrobials in the country; furthermore *S. aureus* is an important source of antimicrobial resistance determinants [9,10]. Therefore this study was aimed to fill these knowledge gaps by investigating prevalence of *S. aureus* nasal carriage in healthy humans, pigs and dogs in Tanzania, by characterizing *S. aureus* using *spa* gene typing and

antimicrobial resistance among human and veterinary isolates.

Methodology

Ethical clearance and human subjects' consent

All human participants were informed of the objective and methods of the study and voluntarily signed an informed consent form. The National Institute for Medical Research of Tanzania (NIMR) approved the study (ethical clearance certificate No 936 dated 01/4/2010 (NIMR/HQ/R.8a/Vol.IX/936). The Ethical Committee- of Sokoine University of Agriculture (SUA) approved the animal sampling study.

Sample collection

Nasal swabs were collected from 100 epidemiologically unrelated healthy humans, 100 pigs and 100 dogs from urban and peri-urban Morogoro Municipality, Tanzania, from December 2011 through March 2012. Adult pigs were sampled at different pens and houses within eight rearing places representative of the study area. A single sterile cotton swab was inserted 1- 2 cm into both nostrils and rotated along the nasal mucosa for 5 -10 s. Swabs were rubbed well by rotating 5 times over the inner wall of the ala and nasal septum. Swabs were stored in sterile tubes containing Cary Blair transport medium and were transported in a cool box to the laboratory.

Isolation and identification of S. aureus

After enrichment in Mueller Hinton Broth (Oxoid Ltd, Basingstoke, UK) containing 6.5% NaCl for 24 hours at 37°C, 10µl of the enrichment culture were streaked on Oxacillin Resistance Screening Agar Base (ORSAB) (Oxoid Ltd, Basingstoke, UK) without selective supplement. Blue colored colonies were harvested and stored at -80°C in Muller-Hinton Broth (Oxoid Ltd) with 50% glycerol. Subsequently, isolates were streaked on blood agar and presumptive staphylococci were tested for coagulase activity in horse plasma. Coagulase-positive isolates were screened by *nuc*-PCR to confirm *S. aureus* [11]. *mecA* [12] and *mecC* PCR were also carried out [13, 14]. All PCR primers used in *nuc, mecA* and *mecC* were obtained from TAG Copenhagen A/S Kong Georgs Vej 12 DK-2000 Frederiksberg in Denmark. Positive controls included *S. aureus* strain ATCC 25923 from the National Medical research Institute Tanzania [15] used for nuc-PCR, *S. aureus* 50A247 for mecA PCR [16] and for *mecC* PCR; *S. aureus* strains 68529-2 and 11-2 were obtained from University of Copenhagen laboratory. Vials without DNA were likewise included as negative controls.

spa typing

All *S. aureus* isolates were *spa* typed and sequenced and cluster analysis based on sequence types were performed using the Ridom StaphType software (Ridom GmbH, Würzburg, Germany) as previously described [17]. The *spa* server database [18] was consulted to extrapolate the *spa* types to previously reported STs or cloned complexes (CC). Alternatively, presumed STs and CCs were assigned based on

Table 1. *spa* type associated STs and *spa* cluster analysis of *S. aureus* isolated from healthy humans, dogs and pigs in Tanzania.

spa type	Associated ST or CC*	*spa* cluster*	Human isolates	Dog isolates	Pig Isolates	Total number of isolates
t084	ST15, ST18		3	1		4
t127	ST1	I		4		4
t131	ST80				4	4
t714	None		4			4
t148	ST72		3			3
t223	ST22		2	1		3
t311	ST5	II	2			2
t314	ST121		1	1		2
t002	ST5, ST231	II	1			1
t015	ST45		1			1
t267	CC97			1		1
t451	ST8	III	1			1
t508	None			1		1
t690	None		1			1
t1476	None	III		1		1
t1849	None	I	1			1
t2030	None		1			1
t10779	New		1			1

* STs and Clusters were assigned based on known associations with *spa* types [13].

associations with *spa* types reported in the scientific literature.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by disc diffusion according to the Clinical and Laboratory Standards Institute (CLSI) [19]. The test was performed on Mueller Hinton agar (Oxoid Ltd) using sulphamethoxazole/ trimethoprim (STX 25 µg), ampicillin (AMP 10 µg), rifampicin (RD 5 µg), amoxycillin-clavulanic acid 2:1 (AMC 30 µg), oxacillin (OX 1 µg), chloramphenicol (C 30 µg), gentamicin (CN 10 µg) and tetracycline (TE 30 µg) discs (Oxoid Ltd.). *S. aureus* ATCC 25923 was included for quality control.

Results

Staphylococcus aureus was isolated from 22/100 humans, 4/100 pigs and 11/100 dogs. None of the strains were positive neither by mecA PCR nor by mecC. The results of *spa* typing of *S. aureus* are summarized in Table 1. Human isolates had 13 distinct *spa* types, including a new *spa* type (t10779). The most frequently found types were t714 (no associated ST reported, n = 4), t148 (ST72, n = 4), t084 (ST15 and ST18, n = 3), t311 (ST5, n = 2) and t223 (ST22, n = 2). Dog isolates showed seven distinct *spa* types with t127 (ST1, n = 5) being the most frequent type. All four *S. aureus* isolates from pigs belonged to t131 (ST80). Human and dog isolates shared three *spa* types: t314 (ST121), t084 (ST15 and ST18) and t223 (ST22).

Results of antimicrobial susceptibility testing are shown in Table 2. All isolates were resistant to ampicillin. Tetracycline resistance was observed in strains isolated from the three host species. No isolates were resistant to oxacillin.

Discussion

The present study provides novel information about carriage frequency and diversity of *S. aureus* in healthy humans, dogs and pigs outside hospital and clinical settings in Tanzania. None of the isolates were positive in PCR targeting the *mecA* and *mecC* genes, indicating that none of the isolates were MRSA positive.

The observed frequency of *S. aureus* isolation from humans (22%) was within the range shown in other studies, where about 20% (range 12%–30%) of individuals are persistent *S aureus* nasal carriers, approximately 30% are intermittent carriers (range 16%–70%), and about 50% (range 16%–69%) are non-carriers [20]. Since this study was designed as a cross-sectional study, we were not able to distinguish between permanent and intermittent carriers.

The prevalence of *S. aureus* carriage in dogs (11%) was in agreement with previous studies reporting isolation frequencies between 9%-12 % in this animal reservoir [21, 5]. Other studies [6] reported *S. aureus* carriers of 5.9%, 17.6% and 17.6% in pharynx, rectum and nasal cavity, respectively. This is valuable information to understand the epidemiology of *S. aureus* in this animal specie [4] as the majority of previous studies in dogs focused on MRSA [21, 22]. Among the STs isolated from dogs, ST1, a lineage associated to humans, was the most frequent. The human epidemic clone ST22, also known as E-MRSA 15, has previously been isolated from dogs in other geographical regions [6,22,23], supporting the notion that this *S. aureus* lineage may have adapted to the canine host.

S. aureus was isolated from 3 out of 8 pig farms and only 4 pigs were carriers. MRSA occurrence in intensive pig farming in Europe and South East Asia is high [3,24], however little is known about occurrence of non MRSA in pigs, not to mention in the semi-outdoor production system used by smallholder pig farmers in Tanzania. This system might offer few opportunities for *S. aureus* to colonize and/or persist on the pigs and in the farm environment as reported in outdoor studies in France/Spain [25]. Epidemiology studies on MRSA in intensive pig farming have shown that once this type of *S. aureus* is present in the farm, it colonizes a large proportion of the pigs [26]. Contrary

Table 2. Antibiotic resistance in isolates (%) of *S. aureus* from Tanzania.

Source	STX	TE	AMP	RD	AMC	OX	C	CN
Humans (n =22)	3 (13.6)	10 (45.5)	22 (100)	1 (4.5)	1 (4.5)	0	2 (9.1)	2 (9.1)
Pigs (n=4)	0	2 (50)	4 (100)	1 (25)	0	0	1 (25)	1 (25)
Dogs (n=11)	0	4 (36.4)	11 (100)	0	0	0	0	0
Total (n=37)	3 (8.1)	16 (43.2)	37 (100)	2 (5.4)	1 (2.8)	0 (0)	3 (8.1)	3 (8.1)

Sulphamethoxazole/ trimethoprim (STX), tetracycline (TE), ampicillin (AMP), rifampicin (RD), amoxycillin-clavulanic acid (AMC), oxacillin (OX), chloramphenicol (C), gentamicin (CN).

to MRSA, the low in-farm prevalence (0%-3%) shown in this study suggests low transmission rates of susceptible *S. aureus*. A longitudinal study showed the highest transmission rates for MRSA in young pigs [26]. Our sample was withdrawn from pigs of approximately 4-6 months of age, and this may have contributed to the low prevalence.

spa typing is routinely used as the golden standard in *S. aureus* epidemiology studies [7]. All the porcine isolates in the current study were of the same *spa* type, t131 and to the best of our knowledge this is the first report of this type in Tanzania; *spa* type t131 has been associated to ST80, an important community-acquired MRSA lineage in humans [27,28] that has previously been associated to zoonotic transmission between domestic animals and humans [29]. The isolation of only one *spa* type in pigs indicates that this lineage is prevalent among the farms in the study area. It should be noted that farmers bought piglets from independent suppliers and did not share veterinarians, feed supply or other common utensils. Thus the four porcine *S. aureus* isolates were apparently not epidemiologically related.

Three *spa* types were common to humans and dogs: t314 (ST121), t084 (ST15 and ST18) and t223 (ST22). Since none of our samples were paired samples from owners and their dogs, it appears that people and dogs share specific *S. aureus* lineages; whether they are maintained in the two hosts by bidirectional transmission remains to be demonstrated. Apart from one new *spa* type (t10779), all other *spa* types detected in the current study have previously been reported elsewhere [18]. *spa* types mentioned above have been reported in other African countries but with isolation from other parts of the body. For example, studies from Nigeria [30] reported these *spa* types in wounds (t127, t314, t331, t084 and t002), otitis media (t084 and t311), blood stream (t084) and urinary tract infection (t311).

All isolates were resistant to ampicillin, often resistant to tetracyclines, but rarely resistant to aminoglycosides, rifampicin, sulfonamides and phenicols. Resistances to ampicillin and tetracycline have been reported worldwide [5], including referral hospitals in Tanzania [31,32] and community settings in other African countries such as Ghana [33]. Resistance to gentamicin was reported in 41.5% of human clinical isolates derived from a hospital in Tanzania [34] but it was low among human and animal isolates investigated by our study. The elevated frequency of tetracycline resistance found in this study (43.2% of the isolates) may be related to the frequent use of this antimicrobial in both animals [35] and humans [8] in Tanzania. The latter study reported a prevalence of 52.4% tetracycline-resistant isolates from wounds, pus, and nasal swabs well corresponding to the 45.5% of resistance observed in nasal swabs from humans in our study. Resistance was generally higher in human isolates than in animal strains.

This study used *spa* typing as the only tool [36] to type the *S. aureus* species, which makes it impossible to draw safe conclusion on the population structure of the clones circulating in Tanzania. Other studies have combined *spa* typing in combination with based upon repeat pattern (BURP) multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) [37]. A similar approach would have strengthened the current study. Also the samples size was small, and we cannot rule out that more types would have been detected, had more samples been analyzed.

Further studies are warranted in Tanzania to cover a larger area and to be more specific in identification and typing the isolates in relation to pets and their owners as well as for pig keepers and their pigs. Cats should also be included in further studies. A combination of *spa* typing and other laboratory tools should be used.

Conclusion

mecA and *mecC* positive isolates were not detected. Humans and dogs were found to carry *S. aureus* with frequencies that are within the range of reports from other countries. Prevalence determined in pigs may be considered low considering the availability of data on the occurrence of MRSA in intensive pig farming in Europe and South East Asia; however, generally little is known on carrier frequency among pigs, alongside with MRSA in Tanzania. Antimicrobial resistance was generally low with the exception of ampicillin and tetracycline. *spa* typing of *S. aureus* revealed specific *spa* types shared by humans and canine isolates, suggesting possible transmission between these two host species. The community-associated lineage ST80 *spa* type t131 was isolated only from pigs. Further studies with a larger collection of strains are needed to determine whether pigs are the sole reservoir or also humans carry this lineage in Tanzania

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Author contributions

Abdul Katakweba took part in the planning, performed sampling and typing, evaluated results and drafted the manuscript. Carmen Espinosa-Gongora collaborated in the analysis of *spa* sequences and in the revision of the manuscript. Luca Guardabassi took part in the revision the manuscript. Amandus Muhairwa took part in the planning and approval of the final manuscript. Madundo Mtambo took part in the planning, evaluated the results and approved the final manuscript. John Olsen took part in planning of the study, evaluated results and took part in drafting and revision of the manuscript.

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