Geographic distribution of *Staphylococcus* spp. infections and antimicrobial resistance among dogs from Gauteng Province presented at a veterinary teaching hospital in South Africa

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Short Title: Spatial distribution of canine staphylococcal infections

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Abbreviations

VTH, Veterinary teaching hospital; SEB, Spatial Empirical Bayesian; AMR, Antimicrobial resistance; MDR, Multidrug resistant resistance; RR, Relative risk; MRSA, Methicillin-Resistant *Staphylococcus aureus*; GIS, Geographic information system; SAS, Statistical Analysis System

Abstract

The objective of this study was to investigate spatial patterns of staphylococcal infections and resistance patterns of clinical isolates from dogs from Gauteng province in South Africa. Data from records of 1,497 dog clinical samples submitted to a veterinary teaching hospital between 2007 and 2012 were used in the study. Spatial empirical Bayesian smoothed risk maps were used to investigate spatial patterns of staphylococcal infections, antimicrobial resistance (AMR), and multidrug resistance (MDR). Moran's I and spatial scan statistics were used to investigate spatial clusters at municipal and town spatial scales. Significant clusters of staphylococcal infections were identified at both the municipal (Relative Risk [RR]=1.71, p=0.003) and town (RR=1.65, p=0.039) scales. However, significant clusters of AMR (p=0.003) and MDR (p=0.007) were observed only at the town scale. Future larger studies will need to investigate local determinants of geographical distribution of the clusters so as to guide targeted control efforts.

1. Introduction

Although *Staphylococcus* spp. are commensals on the skin and mucosal surfaces of dogs, association between colonization and the risk of infection with these organisms has been reported (Beça et al., 2015; Biberstein et al., 1984). *Staphylococcus* spp. are the leading causes of pyoderma, otitis media, and wound infections in companion animals such as dogs (Ganiere et al., 2005; Kroemer et al., 2014; Weese et al., 2006). However, in South Africa, the burden of staphylococcal infections among dogs presented at veterinary hospitals has not been investigated. Prevalence of *Staphylococcus* spp. infections among healthy and clinical dog cases have been shown to vary greatly (Duquette and Nuttall, 2004; Gandolfi-Decristophoris et al., 2013; Ganiere et al., 2005). Moreover, in South Africa, the incidence of *Staphylococcus* spp. among dogs is reported to be on the rise (Daniel N. Qekwana et al., 2017). These findings are of public health significance because transmission of infections from dogs to humans have been reported following exposure to carrier or infected dogs (Boost et al., 2007; Faires et al., 2009; Frank et al., 2010; Guardabassi et al., 2004; Pantosti, 2012; Pompilio et al., 2015).

Of concern is the significantly higher proportion of *S. aureus* and *S. pseudintermedius* isolates resistant to lincosamides, fluoroquinolones and trimethoprim-sulphamethoxazole among dogs in South Africa (Daniel N Qekwana et al., 2017). Another South African study by Blunt et al. (2013) also reported high proportions *S. pseudintermedius* isolates resistant to ampicillin and doxycycline among pyoderma cases in dogs. This is not surprising as resistance to various antimicrobial agents among *S. aureus* and *S. pseudintermedius* in dogs has also been reported in other studies

(Guardabassi et al., 2004; Hoekstra and Paulton, 2002; Prescott et al., 2002; Werckenthin et al., 2001).

Although reports by Qekwana et al. (Daniel N Qekwana et al., 2017; Daniel N. Qekwana et al., 2017) suggest that variations in risks of *Staphylococcus* infections and antimicrobial resistance among the *Staphylococcus* isolates are due, in part, to host factors, it is quite possible that local environmental factors might also play a role. Therefore, studies investigating the spatial epidemiology of *Staphylococcus* spp. infections, antimicrobial resistance (AMR) and multi-drug resistance (MDR) among *Staphylococcus* spp. isolates are needed to help identify the geographic distribution of these infections. This information is useful to help better predict the risks of *Staphylococcus* spp. infections and resistance in both humans and companion animals to guide control efforts (Pfeiffer et al., 2008).

After disease clusters are identified, the information gathered can be used to assess potential factors associated with disease occurrence in identified regions and help develop mitigation strategies. Kulldorff's spatial scan statistics, implemented in SaTScan™ (Kulldorff and others, 2006), has been successfully used in a number of epidemiological studies to detect and evaluate disease clusters (Haddow et al., 2011; Kulldorf, 1999; Kulldorff et al., 1998; Daniel M Saman et al., 2012). Grundmann and co-workers used spatial scan statistics to investigate clustering of methicillin resistant *Staphylococcus aureus* (MRSA) in Europe and reported presence of regional clusters of MRSA isolates in their study region (Grundmann et al., 2010). It is possible that risks of *Staphylococcus* infections, AMR and MDR among *Staphylococcus* spp. isolates in South Africa also exhibit spatial patterns that if identified would guide control efforts. Therefore,

the objective of this study was to investigate spatial patterns of risks of *Staphylococcus* spp. infections, AMR and MDR among *Staphylococcus* isolates from dogs presented at a veterinary teaching hospital in South Africa.

2. Methods

2.1 Study Area

This study was conducted in Gauteng province, located in the Highveld region of South Africa. The province has a subtropical climate with an annual summer rainfall of approximately 700 mm. It has four seasons: summer (November-March), autumn (April-May), winter (June-August) and spring (September-October). It is surrounded by four provinces: Free State province to the South, North-West province to the west, Limpopo to the north, and Mpumalanga to the east. Gauteng has ten administrative municipalities: Ekurhuleni, Emfuleni, Merafong, Midvaal, Mogale City, Randfontein, Westonaria, Johannesburg, Tshwane and Lesedi (Fig. 1). The total number of towns in the province in 2018 was 80. Municipalities, larger than towns, are administrative boundaries used for local government administrative activities. Towns, on the other hand, are settlement areas larger than a village but smaller than a municipality. Thus, several towns comprise a municipality.

The province is estimated to be approximately 18,178 km^2 in size with a population of 12.3 million people with the highest population living in the City of Johannesburg (4,949,347), followed by Ekurhuleni (3,379,104) and City of Tshwane (3,275,152) municipalities. Overall, black Africans comprise the majority (10,770,177) of the population followed by Whites (1,828,849). Forty percent of Gauteng residents have high school education while 13% have higher than the high school education attainment. The unemployment rate among 15-64 year olds in the province is 27.7%. Manufacturing is the main source of employment for the majority of people living in the province, followed by construction, mining, and agriculture. The average annual household income was estimated at R193,771 (USD 19,377) in 2017. On average, 33% of total annual

household expenditure goes to housing, water, electricity, gas and fuel and only 15% to miscellaneous goods and services.

2.2 Data Source and preparation

Laboratory records of clinical samples from dogs from Gauteng Province presented at the University of Pretoria Veterinary Teaching Hospital (VTH) for microbiological diagnosis between January 2007 and December 2012 were included in this study. The VTH is a large and the only referral veterinary teaching hospital in South Africa and is equipped with a modern bacteriology laboratory. The laboratory handles the processing of samples of clinical cases cared for at the hospital. Of the 1,497 samples from Gauteng province, 396 were *Staphylococcus* positive and were included in subsequent analyses. However, antimicrobial susceptibility tests were performed on only 382 *Staphylococcus* positive isolates. The following fields were extracted from each record: residential address, submitted specimen-type, *Staphylococcus* species isolated and antimicrobial susceptibility test results. The data were inspected for inconsistencies such as missing and incorrect addresses, assessed for duplicate entries and if any animals were sampled multiple times during the study period. No duplicates were identified and the dataset did not contain multiple tests from the same patient. There were also no results of mixed infections.

Residential addresses of all patients were geocoded using ArcView GIS10.1 (ESRI Inc., Redlands, California, USA). Point-in-polygon joins were then used to join the geographic coordinates (latitudes and longitudes) of the patients' residence to the town and local municipality shape files. The *Staphylococcus* and antimicrobial resistance data

were then aggregated to these two spatial scales. All spatial analyses were performed at the local municipality (Fig. 1) and town (Fig. 2 and Table 1) spatial scales.

2.3 Data Analysis

The proportions of *Staphylococcus* positive, AMR and MDR isolates were calculated at local municipality and town spatial scales using SAS 9.4 (SAS Institute Inc., Cary, North Carolina, USA) statistical software. At the local municipality and town spatial scales, all geographic units with <1% proportion of samples were aggregated into one group called 'All Others' (see Tables 2 and 3).

The proportions of *Staphylococcus* positive samples and antimicrobial resistance among *Staphylococcus* isolates at the town spatial resolution were smoothed using spatial empirical Bayesian (SEB) smoothing in GeoDa (Anselin et al., 2006) using first-order queen contiguity spatial weights. In small area mapping, spatial empirical Bayesian smoothing adjusts for spatial autocorrelation and non-homogeneity of variance associated with differences in sample sizes across geographic units (in this case, towns) under study (Bernardinelli and Montomoli, 1992; Cuzick and Edwards, 1990; Haddow et al., 2011; Pedigo et al., 2011; Pfeiffer DU, Robinson TP, Stevenson M, Stevens KB, Rogers DJ, 2008; Daniel M. Saman et al., 2012).

2.4 Detection of Spatial Clusters

Global Moran's I was used to assess for spatial clustering of unsmoothed proportions of *Staphylococcus* infections, AMR, and MDR among *Staphylococcus*

isolates at both local municipal and town spatial scales. Significance of Moran's I was assessed using 9999 permutations (Anselin et al., 2006).

Spatial scan statistics (Kulldorff and Nagarwalla, 1995), was used to identify local clusters of *Staphylococcus* spp. infections and AMR at the municipal and town spatial scales. Retrospective purely spatial analysis using Poisson models were used to identify significant purely spatial high risk clusters of *Staphylococcus* infections and AMR. No geographic overlap of the clusters were allowed. The maximum cluster size was left at 50% of the total population at risk and the significance of the clusters was assessed at $\alpha=0.05$ using 9999 Monte Carlo replications. ArcView GIS (version 10.1) was used to display the results of identified clusters. The following information was reported for the identified clusters: location, number of areas (municipalities or towns), numbers of observed and expected cases, relative risk (RR) and p-value.

3. Results

At the local municipal spatial scale, Johannesburg had the highest (43.9%) unsmoothed proportion of *Staphylococcus* positive samples followed by the East Rand local municipality (31.2%) (Table 2, Fig. 3a). Similarly, the results of the SEB smoothing at the town spatial scale showed higher proportions of *Staphylococcus* isolates in western and south-central parts of the province. Although similar patterns were observed at the town spatial scale with the risk ranging from 47% to 56%, this spatial scale provided more detail in spatial heterogeneity than the municipal spatial scale (Fig. 3b).

Resistance to at least one antimicrobial category among *Staphylococcus* spp. isolates at local municipality level was highest in western and south-central areas of the province (Table 3, Fig. 4a). Again, although a similar general spatial pattern is evident at the town spatial scale, a lot more detail and heterogeneity is revealed (Fig. 4b). Multidrug resistance (MDR) among *Staphylococcus* isolates at local municipal spatial scale was highest in the southern region of Gauteng province. Similar patterns of MDR among *Staphylococcus* isolate were observed at town spatial scale although some additional towns with quite high risks are revealed to the north of the study area; these were not revealed at the municipal spatial scale (Fig. 5).

There was evidence of global spatial clustering of *Staphylococcus* infections at both the local municipal spatial scale (Moran's I= 0.342, p=0.006) and the town spatial scale (Moran's I=0.398, p=0.001). However, AMR and MDR did not show evidence of significant global spatial clustering at the municipal scale (Table 4). In contrast, both AMR and MDR showed evidence of significant global spatial clustering at the town spatial scale

(Table 4). Significant local spatial clusters of *Staphylococcus* infections were detected at both the local municipal (p=0.003) and town (p=0.045) spatial scales in the central and south-western regions of the province (Fig. 6). The risk of infection in the municipality local cluster was 1.7 (RR=1.71) times higher than in municipalities outside the cluster. Similarly, the risk within the local cluster identified at the town spatial scale was also approximately 1.7 (RR=1.65) times higher than in towns outside the cluster (Fig. 6).

4. Discussions

This study investigated spatial patterns of canine *Staphylococcus* spp. infections as well as their AMR and MDR patterns with a view to identifying geographic hotspots. Study findings show evidence of clustering of *Staphylococcus* isolates in the Western, Central and Southern regions of Gauteng province. The reasons for these clusters are unclear at this time since the sample size in our dataset did not allow for further detailed investigations of determinants of the identified hotspots. However, we hypothesize that local environmental factors may be responsible for the observed patterns. Suffice it to say that studies in Europe and US on the molecular epidemiology of MRSA have reported regional clustering of *Staphylococcus* cases. In Europe, MRSA compared to MSSA infections clustered in regions in close proximity to hospitals (Grundmann et al., 2010). While in the USA, MRSA infections were more common in the western states compared to other states (Carrel et al., 2015). Unfortunately, no similar studies have been done in South Africa and hence our study provides the first clue regarding the spatial epidemiology of these infections in this region. However, it is important to note that similar to our findings, the authors of the two studies above could not provide the reasons for clustering of MRSA infections.

We hypothesize that clustering of *Staphylococcus* isolates in the current study could be due to socioeconomic and environmental factors (Li et al., 2013; Onozuka and Hagihara, 2007). There is evidence that *Staphylococcus* species can survive for months on environmental surfaces which act as sources of infections to susceptible dogs (Neely, Maley 2000, Coughenour, Stevens & Stetzenbach 2011). Due to the poorer living conditions in low socioeconomic areas, these contaminated environmental surfaces are

expected to be more common in these low socioeconomic areas than in the more affluent areas. This, together with the limited access/utilization of veterinary services in the low socioeconomic areas, may result in clustering of *Staphylococcus* infections in these areas. This is expected to translate into higher burden of diseases among dogs in these areas than those from more affluent areas. Moreover, studies have reported higher risk of *Staphylococcus* spp. infections in dogs with underlying clinical conditions than those with no underlying clinical conditions (Cohn and Middleton, 2010; Kawakami et al., 2010; Kramer et al., 2006; Weese and van Duijkeren, 2010). It is also possible that the clustering of *Staphylococcus* infections may be due to differences in dog care/management strategies such as fencing of household dogs and vaccinations that could play a role in the distribution of *Staphylococcus* species (Daniel N. Qekwana et al., 2017). However, more research needs to be done to investigate factors responsible for the observed patterns.

The results of the spatial scan statistics show no evidence of clustering of resistant isolates at local municipal and town spatial scales. This may suggest that there is spatial homogeneity in antimicrobial resistance among *Staphylococcus* isolates in this study (Kulldorff, 1998; Pfeiffer et al., 2008) probably due to similarity in prescription practices among veterinarians in the areas under study. However, since this is the first study that investigated spatial patterns of antimicrobial resistance among *Staphylococcus* isolates in veterinary medicine in this region, more studies will need to be done using information from other veterinary clinics in these regions to gain better understanding of the spatial epidemiology of antimicrobial resistance.

This study is not without limitations. The samples used in this study were from one veterinary teaching hospital which is not the only veterinary hospital or clinic in Gauteng Province. Therefore, the findings of this study should be interpreted with caution since they may not be representative of all veterinary laboratories or veterinary hospitals in the province. It is possible that not including samples from other veterinary laboratories in the province could affect the distribution of *Staphylococcus* infection and antimicrobial resistance patterns of the isolates from canine clinical cases. In addition, the population under study did not include outpatient cases. This could have resulted in lower proportion of *Staphylococcus* isolates reported in this study. Moreover, clinical canine cases that respond to treatments are often not cultured or sent for antibiogram. Therefore, it is possible that a large population of dogs that responded to empirical treatments were not included in this study. Although the methods used in the study adjusted for the small number problems, it is desirable to have larger sample sizes to improve precision of estimates. Therefore, future studies will need to include more samples from both the veterinary teaching hospital and other veterinary clinics in Gauteng Province. Finally, the use of antibiotics could have resulted in lower recovery rates of *Staphylococcus* species. Unfortunately, this information was not available for inclusion in the study.

5. Conclusions

The above limitations notwithstanding, this study provides useful baseline information on the spatial distribution of *Staphylococcus* infections, AMR and MDR among dogs in Gauteng Province presented at the veterinary teaching hospital. There is evidence that the infections cluster in certain local municipalities and towns. Future

studies will need to investigate determinants of these spatial patterns so as to provide information to guide control efforts.

Authors' contributions

DNQ was involved in study design and data management and performed all statistical analyses and interpretation as well as preparation of the manuscript draft. AO was involved in study design, data analysis and interpretation as well as extensive editing of the manuscript. JWO was involved in study design and editing of the manuscript. All authors have read and approved the final manuscript.

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Ethical considerations

This study was approved by ethics committee of the University of Pretoria (V051-14).

Consent for publication

The study does not involve human subjects and therefore no consent was required.

Conflict of interest

The authors declare that they have no competing interests.

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Table 1: Legend for Fig. 1 showing the identification codes and names of each of the town included in this study.

Town	Code	Town	Code
Alberton	AL	Westonaria Rural	WER
Benoni	BE	Cullinan	CU
Boksburg	BO	Refilwe	RE
Brakpan	BR	Rayton	RY
Bronkhorstspruit	BS	Soshanguve	SO
Kungwini Rural	KR	Akasia	AK
Centurion	CT	Winterveldt	WI
Nokeng tsa Taemane Rural	NT	Lethabong (Ekurluleni)	LTE
De Deur/Walkerville	DD	Tembisa	TB
Devon	DE	Olifantsfontein	OL
Ekurhuleni Rural	EKR	Kempton Park	KP
Eikenhof	EI	Johannesburg Rural	JHR
Ekangala	EK	Soweto	SW
Ga Rankuwa	GR	Midrand	MD
Germiston	GE	Diepsloot	DP
Hammanskraal	HM	Lethabong (City of JHB)	LTJ
Heidelberg - GP	HGP	Roodepoort	RP
Lesedi Rural	LR	City of Johannesburg Rural	CJR
Vaal Marina	VM	Johannesburg South	JS
Vaal Oewer	VO	Randburg	RG
Emfuleni Rural	EM	Alexandra	ALE
Vereeniging-Kopanong (GT421)	VKA	Sandton	SDT
Vereeniging-Kopanong (GT422)	VKB	Mabopane	MB
Krugersdorp	KR	Sebokeng	SB
Mogale City Rural	MGR	Evaton	EV
Magaliesberg	MA	Lenasia	LA
Midvaal Rural	MR	Lenasia South	LAS
Nigel (East Rand)	NE	Ennerdale	ED
Nigel (GT423)	NI	Orange Farm	OF
Pretoria	PR	Lawley	LW
City of Tshwane Rural	CTR	Vanderbijlpark	VP
Randfontein	RF	Kudube	KD
Randfontein Rural	RFR	Diepkloof	DPF
Roodeplaat	RD	Johannesburg	JH
Springs	SP	Meadowlands East	ME
Sterkfontein Rural	SR	Meadowlands West	MW
Temba	TE	Pimville	PV
Vischkuil	VI	Muldersdrift	MDT
Westonaria	WE	Vosloorus	VOS
Tokoza	TK	Katlehong	KAT
Kwamahlanga	KH		

Table 2: Distribution of *Staphylococcus* infections based on clinical samples tested at the bacteriology laboratory of a veterinary teaching hospital, 2007-2012.

	Samples processed		*Staphylococcus* positive samples	
	Number	**Percent**	**Number**	**Percent**
Local municipality	n=1,497[a]		n=396[b]	
Pretoria	1242	83.0	308	24.8
Johannesburg	98	6.6	43	43.9
East Rand	77	5.1	24	31.2
Cullinan	32	2.1	7	21.9
Bronkhorstspruit	20	1.3	6	30.0
All Others	28	1.9	8	28.6
Towns	n=1,497[a]		n=396[b]	
Pretoria	974	65.1	247	25.4
Akasia	129	8.6	33	25.6
Centurion	58	3.9	11	19.0
City of Tshwane Rural	43	2.9	10	23.3
Randburg	23	1.5	10	43.5
Kungwini Rural	20	1.3	6	30.0
Roodepoort	20	1.3	10	50.0
Kempton Park	17	1.1	7	41.2
Roodeplaat	17	1.1	5	29.4
Germiston	15	1.0	8	53.3
All Others	181	12.1	49	27.1

[a] A total of 1,497 samples were tested for presence of *Staphylococcus*
[b] A total of 396 of the 1,497 samples were *Staphylococcus* positive

Table 3: Geographical distribution of antimicrobial resistance patterns among *Staphylococcus* spp. isolated from canine clinical samples tested at the bacteriology laboratory of a veterinary teaching hospital, 2007-2012.

	***Staphylococcus* spp.**		**Antimicrobial Resistance**		**Multidrug Resistance**	
	Number	Percent	Number	Percent	Number	Percent
Local municipality	n=382[a]		n=308[b]		n=223[c]	
Pretoria	296	77.5	232	78.4	161	54.4
Johannesburg	43	11.3	40	93.0	34	79.1
East Rand	22	5.8	19	86.4	14	63.6
Cullinan	7	1.8	6	85.7	5	71.4
Bronkhorstspruit	6	1.6	5	83.3	3	50.0
Meyerton	4	1.1	4	100.0	4	100.0
Randfontein	2	0.5	2	100.0	2	100.0
Vereeniging	1	0.3	0	0.0	0	0.0
Westonaria	1	0.3	0	0.0	0	0.0
Towns	n=382[a]		n=308[b]		n=223[c]	
Pretoria	237	62.0	182	76.8	131	55.3
Akasia	31	8.1	25	80.7	15	48.4
Centurion	11	2.9	10	90.9	6	54.6
Tshwane Rural	10	2.6	10	100.0	7	70.0
Randburg	10	2.6	7	70.0	6	60.0
Roodepoort	10	2.6	10	100.0	7	70.0
Midrand	9	2.4	9	100.0	9	100.0
Germiston	8	2.1	8	100.0	5	62.5
All Others	56	14.7	47	83.9	37	66.1

[a] A total of 382 of the *Staphylococcus* isolates were subjected to antimicrobial susceptibility test

[b] A total of 308 of the 382 isolates subjected to antimicrobial susceptibility tests were resistant to at least one antimicrobial

[c] A total of 223 of the 382 isolates subjected to antimicrobial susceptibility tests were multidrug resistant

Table 4: The results of the global Moran's I spatial autocorrelation tests on proportion of *Staphylococcus* infections, antimicrobial resistance and multidrug resistance in Gauteng Province, South Africa, 2007-2012.

	Local municipalities		Towns	
	Moran's I	**P-value**	**Moran's I**	**P-value**
Staphylococcus infection	0.342	0.006	0.398	0.001
Antimicrobial resistance	0.209	0.094	0.356	0.003
Multidrug resistance	0.232	0.055	0.303	0.007

Fig. 1. Map of South Africa showing location of the study area: (a) Gauteng Province and (b) local municipalities of Gauteng Province.

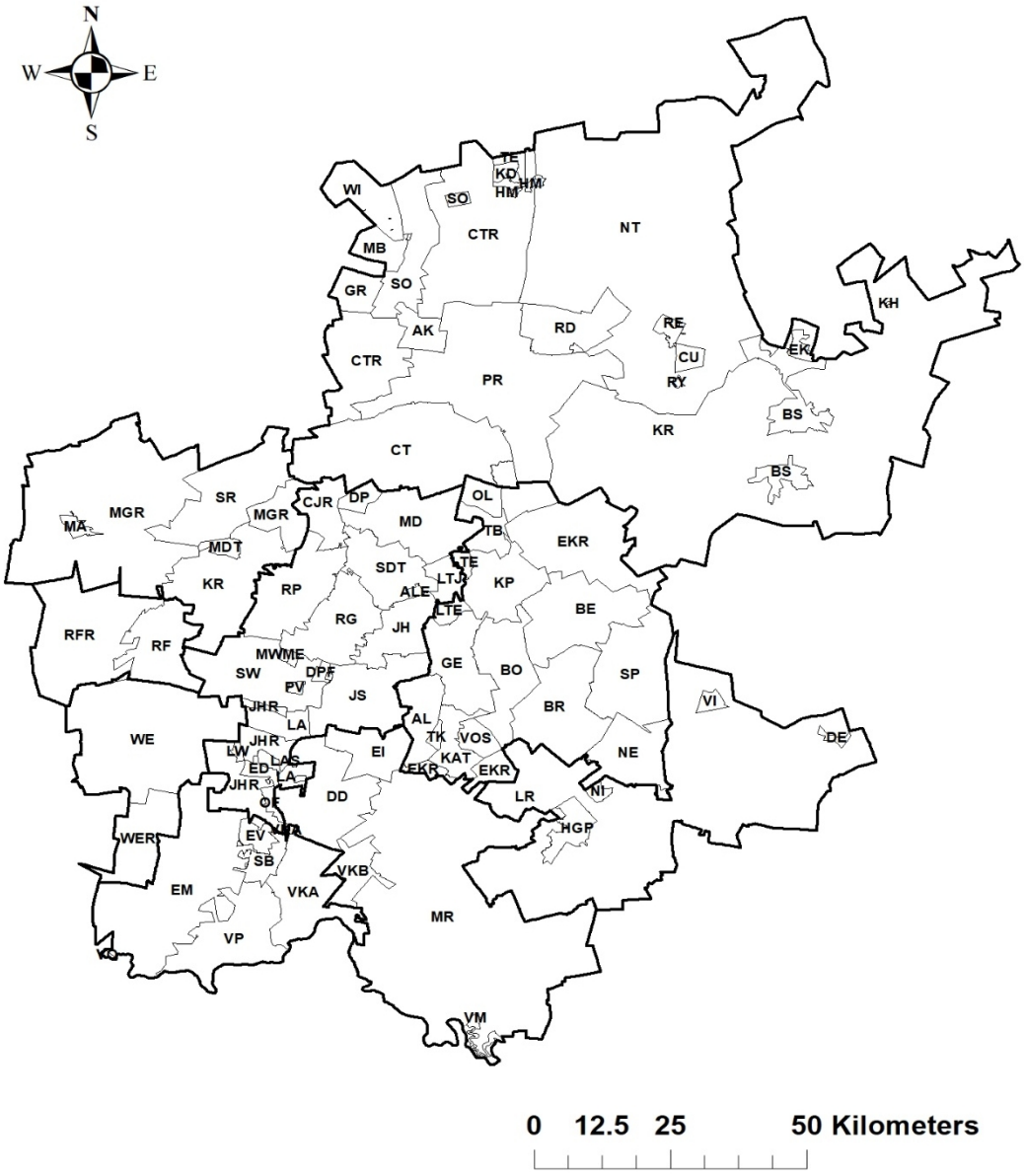

Fig. 2. Map showing distribution of towns of Gauteng Province (See legend on Table 1)

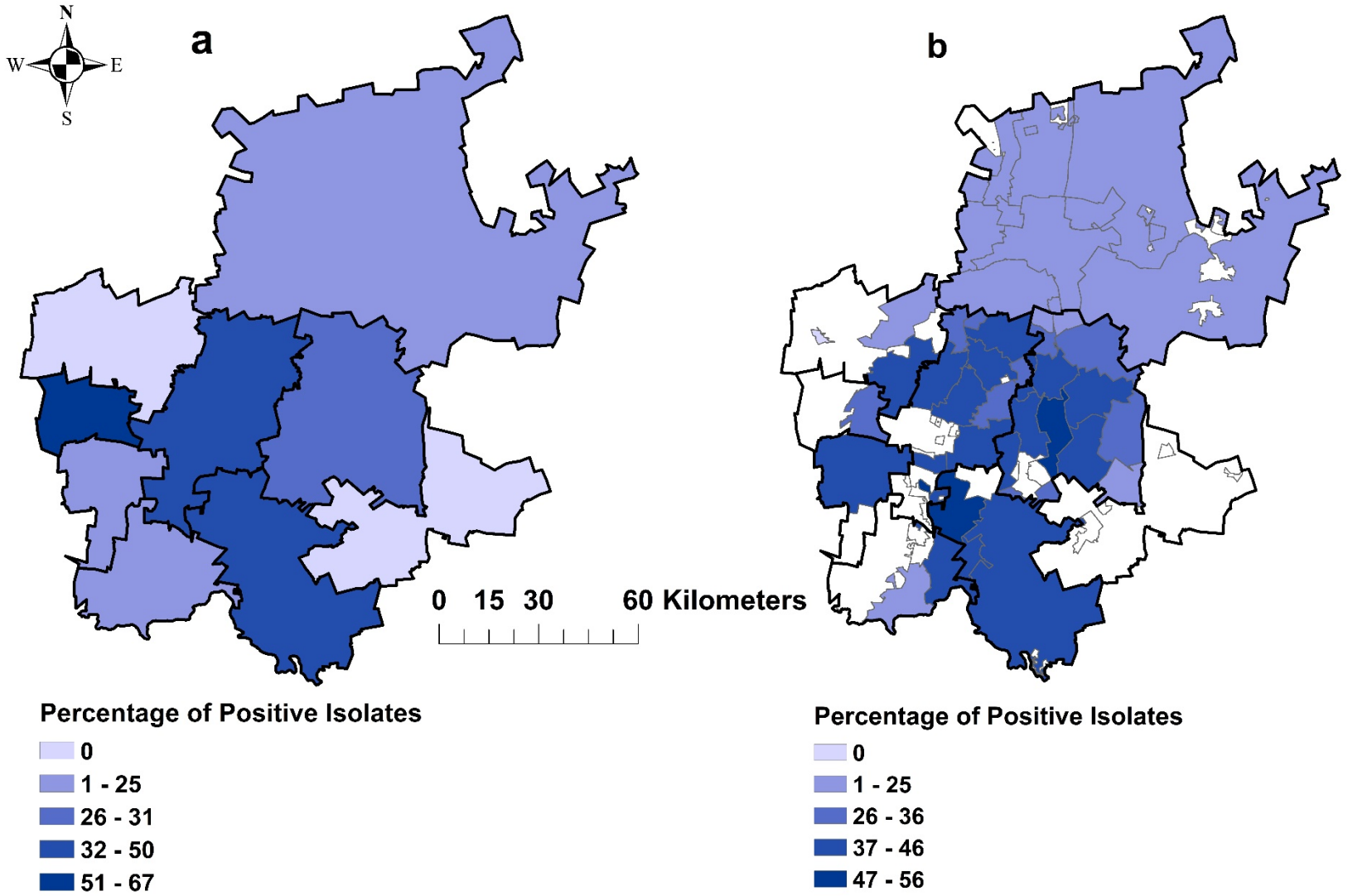

Fig. 3. Geographic distribution of the percentage of *Staphylococcus* positive samples at: (a) municipality and (b) town spatial scales in Gauteng Province, South Africa (2007-2012)

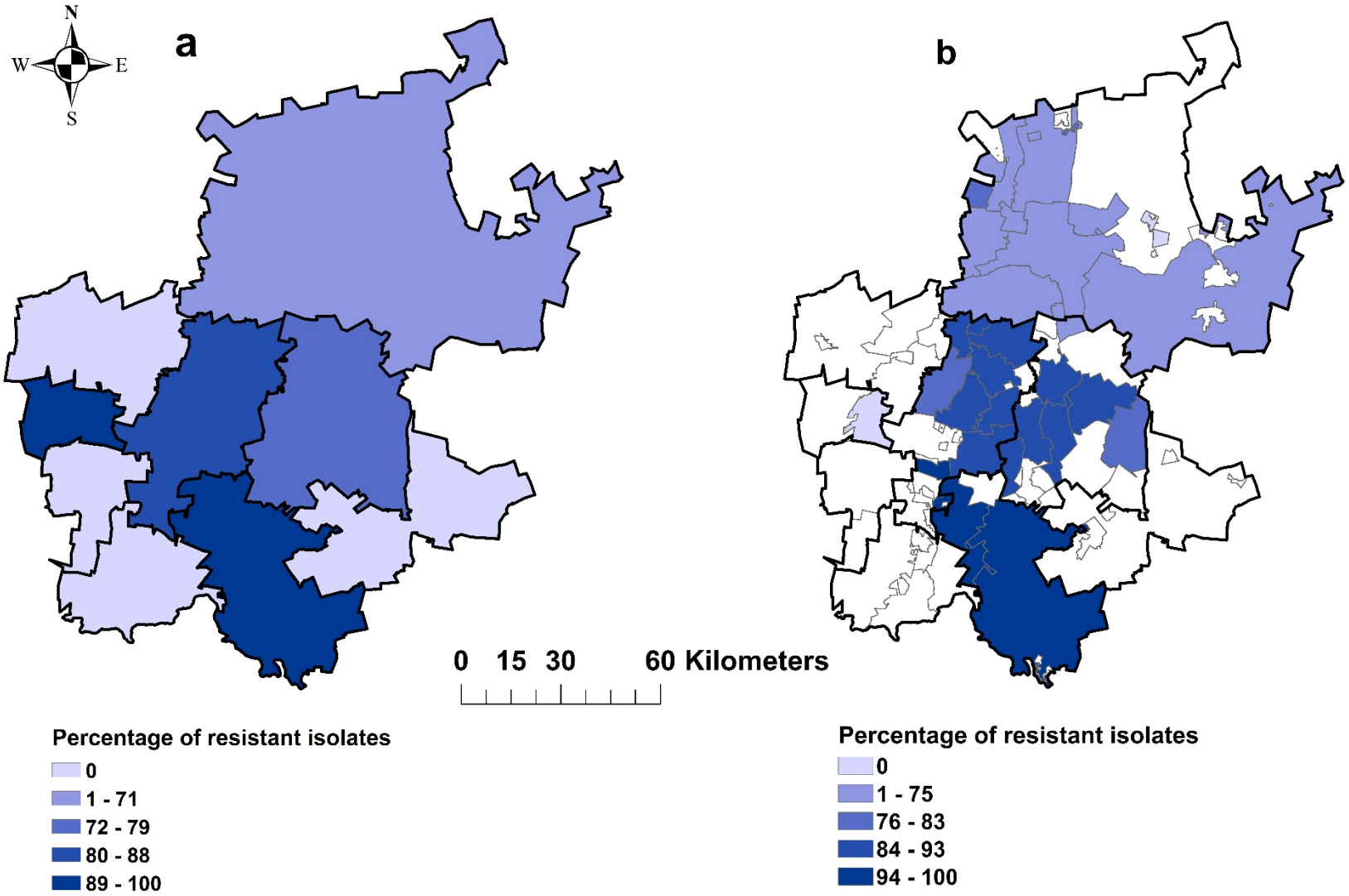

Fig. 4. Geographic distribution of the percentage of antimicrobial resistant *Staphylococcus* isolates at: (a) municipality and (b) town spatial scales in Gauteng Province, South Africa (2007-2012).

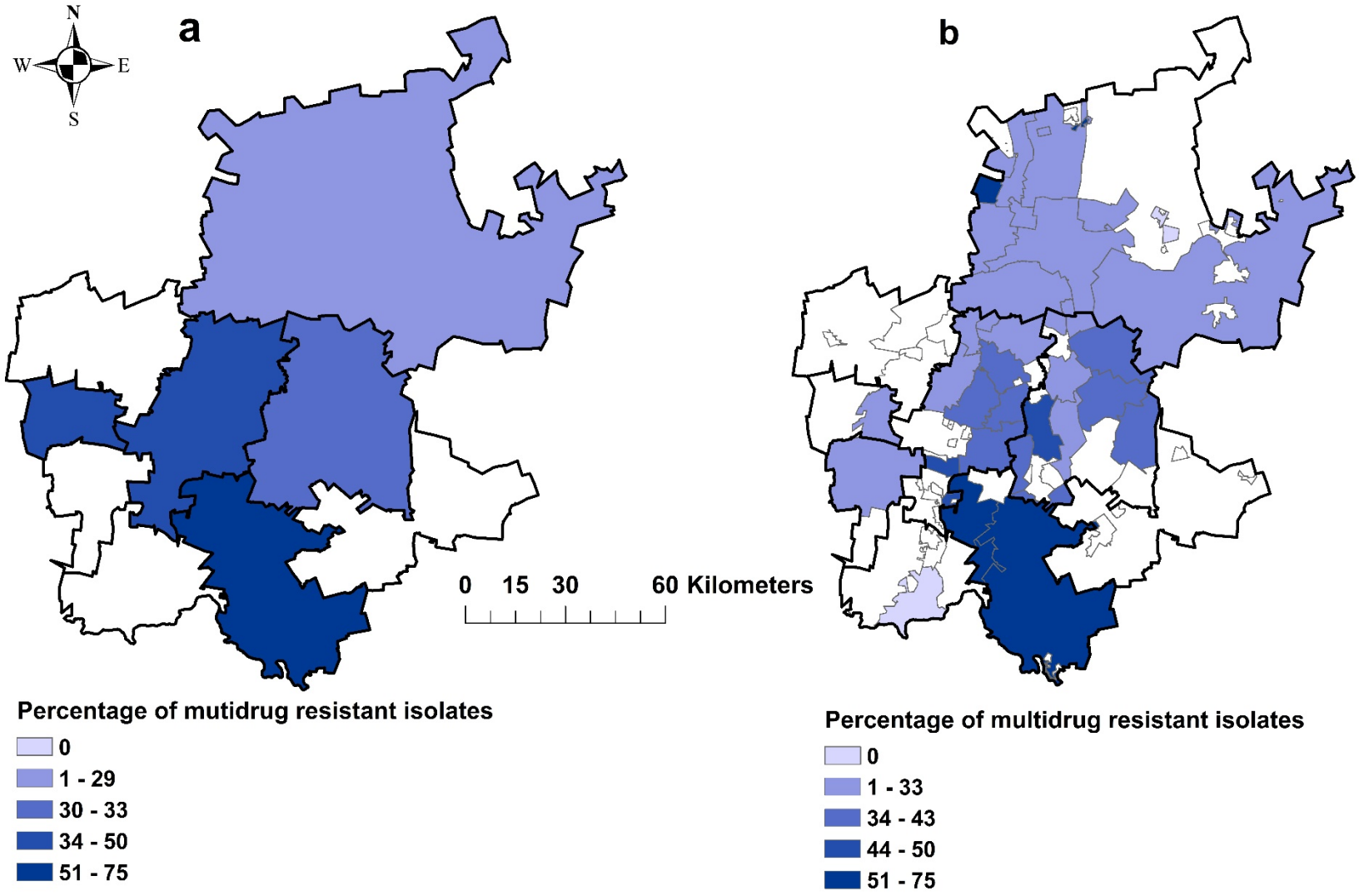

Fig. 5. Geographic distribution of the percentage of multi-drug resistant *Staphylococcus* isolates at (a) municipality and (b) town spatial scales in Gauteng Province, South Africa (2007-2012).

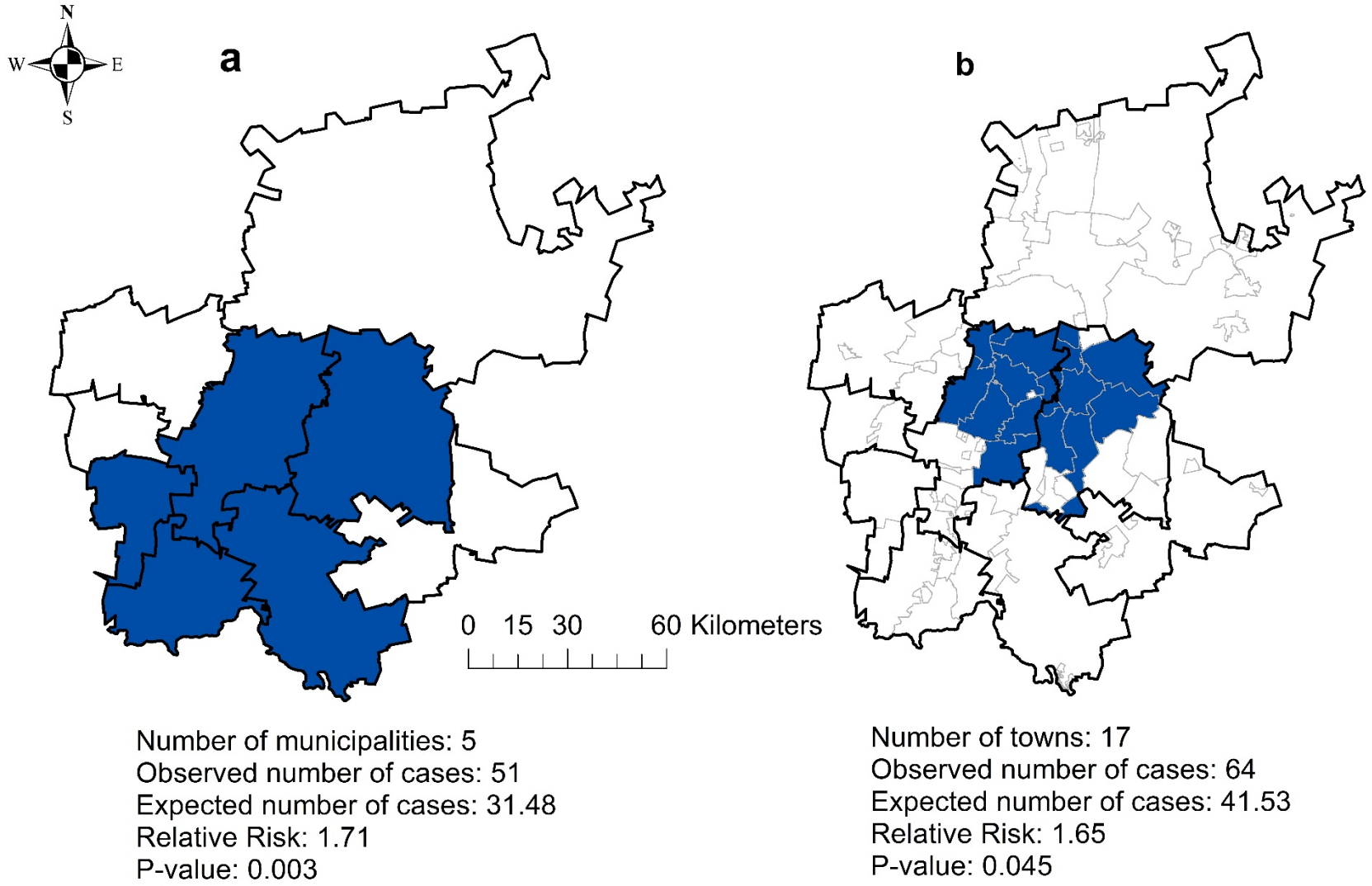

Fig. 6. Spatial clusters of *Staphylococcus* isolates at (a) municipality and (b) town spatial scales in Gauteng Province, South Africa (2007-2012).