

# Time-based distribution of *Staphylococcus saprophyticus* pulsed field gel-electrophoresis clusters in community-acquired urinary tract infections

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*The epidemiology of urinary tract infections (UTI) by Staphylococcus saprophyticus has not been fully characterised and strain typing methods have not been validated for this agent. To evaluate whether epidemiological relationships exist between clusters of pulsed field gel-electrophoresis (PFGE) genotypes of S. saprophyticus from community-acquired UTI, a cross-sectional surveillance study was conducted in the city of Rio de Janeiro, Brazil. In total, 32 (16%) female patients attending two walk-in clinics were culture-positive for S. saprophyticus. Five PFGE clusters were defined and evaluated against epidemiological data. The PFGE clusters were grouped in time, suggesting the existence of community point sources of S. saprophyticus. From these point sources, S. saprophyticus strains may spread among individuals.*

Key words: *Staphylococcus saprophyticus* - PFGE genotype - UTI epidemiology

*Staphylococcus saprophyticus* is an important cause of urinary tract infection (UTI) in young and sexually active women. The source of this organism for human infection has not been completely characterised, although foodborne transmission has been hypothesised (Coton et al. 2010, Leroy et al. 2010, Rall et al. 2010, Soares et al. 2011). Strain typing methods have been used to elucidate the epidemiology of many infectious diseases. However, the genetic relatedness of strains must be validated in epidemiological studies, such as outbreak investigations, and this approach has not yet been applied to *S. saprophyticus* (Widerström et al. 2012). As yet, little is known about the distribution of *S. saprophyticus* genotypes in UTI (Widerström et al. 2012). Few English-language studies have typed collections of *S. saprophyticus* by pulsed field gel electrophoresis (PFGE) and none of these studies were supported by epidemiological data. In a large study with isolates from women with uncomplicated UTI, the PFGE genotypes of 76 isolates collected in Northern Sweden from 1995-1997 were compared with 50 isolates obtained from five different locations in Northern Europe in 2006 (Widerström et al. 2007). Ten PFGE genotypes, including those with up to three band

differences and similarities (Dice coefficient) of 85-100%, were identified. Moreover, the profiles of isolates from various geographic locations were indistinguishable. In another study, 57 *S. saprophyticus* isolates were typed by PFGE: 50 from food and seven from clinical specimens (Coton et al. 2010). No similarities between the food and clinical isolates or among the clinical isolates were observed.

The primary aim of the present study was to determine if epidemiological relationships could be identified between clusters of PFGE genotypes in a collection of *S. saprophyticus* isolates from community-acquired UTI (Dias et al. 2009).

## SUBJECTS, MATERIALS AND METHODS

*Study design* - A cross-sectional surveillance for community-acquired UTI was conducted during March-November 2005 and March-November 2006 among female patients attending two walk-in clinics in the city of Rio de Janeiro (RJ), Brazil. Outpatients with a symptomatic UTI caused by one or two uropathogens, as detected in urine cultures on cystine-lactose-electrolyte-deficient agar medium (Merck, Darmstadt, Germany), at a concentration of  $> 10^3$  colony-forming unit/mL, were eligible for inclusion in the study. Patients who agreed to participate signed a consent form and completed a questionnaire containing demographic and socio-economic information and clinical data. Data from patients with *Escherichia coli*-positive UTI from one of these clinics were reported previously (Dias et al. 2009). In the present study, the clinical and epidemiological characteristics of patients with UTI caused by *S. saprophyticus* and other agents were compared by a chi-square test, Fisher's

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exact test or t-test using the statistical program Epi Info, version 3.5.1 (cdc.gov). A two-tailed  $p \leq 0.05$  was defined as statistically significant. The study was approved by the University Hospital of the Federal University of Rio de Janeiro Institutional Review Board.

**Microbiology methods** - Five colonies of each isolate on a primary growth plate were saved, when possible, for cultures in which *S. saprophyticus* was suspected. The identification of the isolates was confirmed by phenotypic tests (Bannerman & Peacock 2007).

**Strain typing** - *S. saprophyticus* isolates were typed by PFGE with *Sma*I (Roche) as described previously (Rabello et al. 2005). Fragments were separated in a 1% agarose gel in a CHEF-DRIII system (Bio-Rad) with pulse times increasing from 2-35 s over 21 h at 13°C at a voltage gradient of voltage gradient of 6 V/cm. Electrophoretic band patterns were analysed by Gel Compar II version 4.0 (Applied Maths, Kortrijk, Belgium). A Dice coefficient with 1% tolerance and 1% optimisation settings was used for the similarity matrix and the unweighted pair group method with arithmetic mean was used to construct dendrograms.

## RESULTS

**Study population and patient characteristics** - The study included 204 women with a symptomatic, culture-positive UTI. Of these, 32 (16%) patients had UTI caused by *S. saprophyticus*. The patients were mostly from low income backgrounds. Compared with those with UTI caused by other agents, women with *S. saprophyticus* infections were significantly younger, less likely to be pregnant, have had previous UTI or have used antibiotics in

the previous month and more likely to have cystitis (as opposed to pyelonephritis). The clinical and epidemiological characteristics of these patients are presented in Table.

**PFGE typing** - A single colony was obtained from seven (22%) of the 32 patients with *S. saprophyticus*. Multiple colonies (4 or 5) were obtained from each of the other 25 (78%) patients; in total, 118 colonies were saved. Initially, the PFGE profiles of the multiple colonies from the 25 patients were analysed to determine the level of similarity among the members of the *S. saprophyticus* population present in a single patient. Each PFGE profile had 10-14 bands ranging from 90-920 bp. Most of the multiple *S. saprophyticus* colonies belonging to the same patient presented indistinguishable PFGE band patterns (in 22 patients) or included a single different band (in 3 patients). Therefore, for other analyses, the PFGE band patterns considered indistinguishable or that included one band difference were included in a single genotype. Examples of PFGE band patterns of multiple colonies from the same patient are shown in Fig. 1.

Next, the PFGE band patterns of *S. saprophyticus* from all 32 patients were analysed (1 colony of each isolate per patient) and the genotypes were then determined. A total of 18 isolates had unique genotypes, while 14 isolates formed clusters containing two-four isolates each. Three of the clusters contained isolates with indistinguishable profiles, while two clusters contained isolates with a single different band. The dendrogram revealed that the band profiles within the genotype comprised of isolates with up to one band difference shared > 91% similarity. The isolates not included in clusters had < 84% similarity and > three band differences (Fig. 2).

TABLE  
Characteristics of women with urinary tract infection (UTI)  
caused by *Staphylococcus saprophyticus* compared to other bacteria

Characteristic <sup>a</sup>	<i>S. saprophyticus</i> (n = 32) n (%)	Other bacteria (n = 172) n (%)	p
Age in years, median (range)	25 (16-49)	30 (1-86)	0.005 <sup>d</sup>
Black ethnicity <sup>b,c</sup>	19 (59)	100 (58)	0.9
Yearly income <sup>b</sup> (USD)			
≤ 2.483.00	4 (12)	28 (16)	0.7
2.484.00-4.955.00	10 (31)	60 (35)	0.8
4.956.00-10.595.00	9 (28)	49 (28)	0.8
10.596.00-21.180.00	4 (12)	15 (9)	0.7
> 21.180.00	0	5 (3)	0.7
Previous UTI	12 (37)	114 (66)	0.004 <sup>d</sup>
Pregnancy	1 (3)	12 (7)	0.6
Cystitis	27 (84)	108 (63)	0.03 <sup>d</sup>
Antibiotic use	13 (40)	91 (53)	0.3
Previous month	2 (6)	39 (23)	0.05 <sup>d</sup>
Previous six months	11 (34)	52 (30)	0.7

a: data are number and frequency of patients unless otherwise indicated; b: information missing for some patients; c: non-black patients were all white; d: significant differences ( $\leq 0.05$ ); USD: United States dollar.

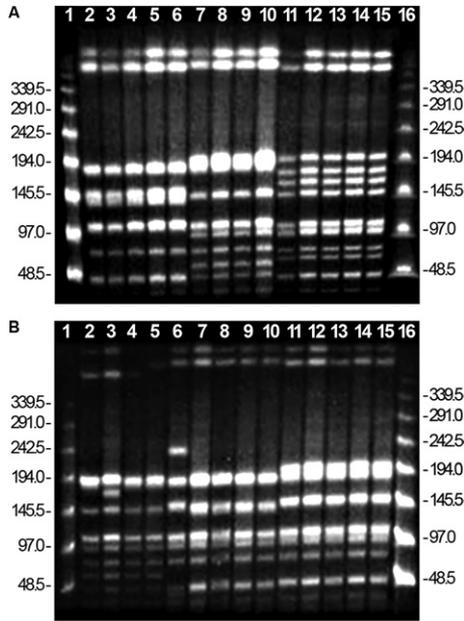


Fig. 1: pulsed field gel electrophoresis profiles of multiple colonies of *Staphylococcus saprophyticus* from patients with community-acquired urinary tract infection. A: Lanes 2-6: isolates from patient "a"; 7-10: isolates patient "b"; 11-15: isolates patient "c"; B: Lanes 2-5: isolates patient "d"; 6-10: isolates patient "e"; 11-15: isolates patient "f".

*Temporal and geographical analysis of PFGE types* - An analysis of the distribution of the PFGE genotypes as a function of time revealed that most of the clusters (4 of 5) occurred within a short time period (3-5 months). Two isolates included in the fifth cluster were obtained in the same year (2006), although they were separated by an interval of eight months (Fig. 3). Therefore, clusters of isolates with the same genotype were also grouped in time. The other genotypes were distributed along the entire study period. An analysis of geographical distribution revealed a dispersal of patients with isolates of the same genotype (data not shown). Although patients were cared for in a single outpatient clinic, they lived in different locations in RJ or outside of the city. Neither clinical nor epidemiological characteristics were significantly different between patients harbouring isolates belonging to PFGE clusters and patients harbouring isolates with unique genotypes. No other associations were identified (data not shown).

**DISCUSSION**

In the present study, the PFGE band profiles of four-five colonies from each of 25 patients with confirmed UTI were compared. It has been suggested that for bacteria, isolates with up to four PFGE band differences could be included within the same genotype; however,

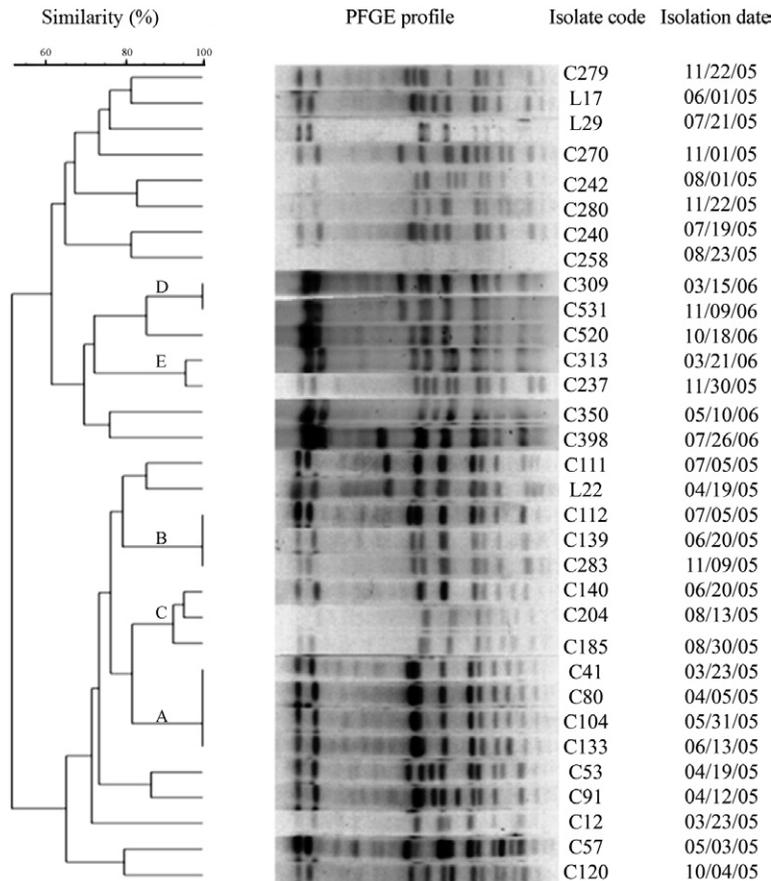


Fig 2: dendrogram of *Staphylococcus saprophyticus* pulsed field gel electrophoresis (PFGE) profiles. Capital letters in the dendrogram refer to genotypes with more than one isolate.

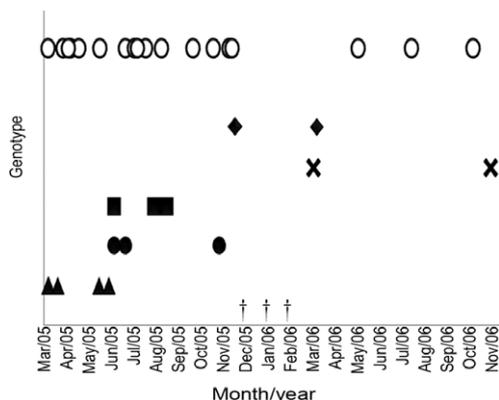


Fig. 3: temporal distribution of *Staphylococcus saprophyticus* pulsed field gel electrophoresis (PFGE) genotypes. Black symbols are genotypes in clusters. Open symbols are the various unique PFGE genotypes. †: data not collected.

depending on the organism, a single band difference could represent a different genotype (van Belkum et al. 2007). Thus, the appropriate definition of a genotype must be validated empirically by epidemiological data, preferably by an analysis of outbreak situations. As *S. saprophyticus* infections are not expected to occur in outbreaks, we chose to investigate diversity within multiple colonies from a single patient and observed that one different band could occur within those colonies. Our data demonstrated that UTI by *S. saprophyticus* is primarily a monoclonal infection. The definition of a PFGE genotype as band profiles with no more than one difference may be too strict; however, it is unlikely that isolates that truly belong to different genotypes are grouped when this definition is used.

We observed clustering over time of isolates of the same PFGE genotype. One limitation of the present study was the small number of isolates studied. However, PFGE clusters were evident and with a larger study sample size, more clusters could be identified. The present findings indicate that possible point source outbreaks of *S. saprophyticus* are likely to occur in our community. This observation would be consistent with the spread of this organism by food vehicles. In addition, a food source that is distributed throughout RJ could explain the geographic dispersion of the isolates of the same genotype. Thus, clustering by PFGE genotype and time is epidemiologically plausible. Indeed, *S. saprophyticus* was recently isolated from fresh cheese in Brazil (Rall

et al. 2010). Unfortunately, there was no molecular comparison of the isolates in that study with clinical isolates that could support fresh cheese as a source of *S. saprophyticus* UTI. Such a hypothesis could be tested only in a longitudinal study.

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