International Journal of Infectious Diseases 81 (2019) 52–56

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Streptococcus pyogenes infections with limited *emm*-type diversity in the homeless population of Brussels, 2016–2018

Nicolas Dauby[a,j,*], Véronique Yvette Miendje Deyi[b], Valérie Delforge[c], Delphine Martiny[b,d], Leila Mekkaoui[b], Marie Hallin[b], Romain Mahieu[e], Nathalie Bossuyt[f], Anne Botteaux[c], Pierre R. Smeesters[c,g,h,i]

[a] *Centre Hospitalier Universitaire Saint-Pierre, Université Libre de Bruxelles (ULB), Brussels, Belgium*
[b] *Laboratoire Hospitalier Universitaire de Bruxelles – Universitair Laboratorium Brussel (LHUB-ULB), Brussels, Belgium*
[c] *Molecular Bacteriology Laboratory, Université Libre de Bruxelles (ULB), Brussels, Belgium*
[d] *Université de Mons (UMONS), Mons, Belgium*
[e] *Hygiene Inspection, Comission Communautaire Commune, Brussels-Capital Region, Brussels, Belgium*
[f] *Epidemiology of Infectious Diseases, Sciensano, Brussels, Belgium*
[g] *Queen Fabiola Children University Hospital, Université Libre de Bruxelles (ULB), Brussels, Belgium*
[h] *Murdoch Children's Research Institute, Melbourne, Australia*
[i] *Centre for International Child Health, University of Melbourne, Melbourne, Australia*
[j] *Centre for Environmental Health and Occupational Health, School of Public Health, Université Libre de Bruxelles (ULB)*

ARTICLE INFO

Article history:
Received 16 November 2018
Received in revised form 15 January 2019
Accepted 17 January 2019
Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Streptococcus pyogenes
Epidemiology
Streptococcal m protein
Skin infections
Homeless persons

ABSTRACT

Objectives: The aim was to characterize the clinical features, outcomes, and strain diversity of laboratory-confirmed *Streptococcus pyogenes* (group A *Streptococcus*, GAS) infections among inpatients hospitalized at a tertiary level hospital in Brussels, Belgium, according to the patients' housing status (homeless vs. not homeless).
Methods: Between August 2016 and January 2018, all patients hospitalized with a laboratory-confirmed GAS infection were prospectively enrolled and risk factors were recorded. GAS strains were characterized using *emm*-typing and *emm*-clustering in both inpatients and outpatients. Analyses were performed according to homelessness status.
Results: During the study period, 48% (28/58) of adults hospitalized with a GAS infection at the tertiary hospital were homeless. The estimated incidence rate was 100 times higher for homeless persons. Skin abscesses were more frequent in the homeless group (21.4% vs. 3.3%) and mortality was high (10.7%). Limited *emm*-type diversity was found in this group, with four *emm*-types (64, 77, 83, and 101) accounting for 76.1% of the infections, and the majority of these *emm*-types belonged to the D4 *emm*-cluster. Pooled analyses of inpatient and outpatient strains indicated lower diversity in the homeless group.
Conclusions: The homeless are disproportionately affected by GAS and have a higher rate of abscesses and high mortality. The lower *emm*-type diversity and preferential infection with four *emm*-types likely reflects endemic circulation of GAS in this population. Preventive strategies are warranted in this fragile population.

Introduction

In recent years, outbreaks of *Streptococcus pyogenes* (group A *Streptococcus*, GAS) infections have been reported in homeless populations across Europe and North America (Cady et al., 2011; Bundle et al., 2017; Mosites et al., 2018). These outbreaks were mostly caused by single *emm*-types and included a high proportion of invasive infections.

A high proportion of homelessness was observed among adults hospitalized with a GAS infection between August 2016 and

* Corresponding author at: Department of Infectious Diseases, Centre Hospitalier Universitaire Saint-Pierre, Rue Haute, 322, 1000 Brussels, Belgium.
E-mail addresses: Nicolas.dauby@ulb.ac.be (N. Dauby), Veronique.MIENDJEDEYI@lhub-ulb.be (V.Y. Miendje Deyi), valerie.delforge@ulb.ac.be (V. Delforge), delphine.martiny@lhub-ulb.be (D. Martiny), leila.mekkaoui@lhub-ulb.be (L. Mekkaoui), marie.hallin@lhub-ulb.be (M. Hallin), rmahieu@ccc.brussels (R. Mahieu), Nathalie.Bossuyt@sciensano.be (N. Bossuyt), Anne.Botteaux@ulb.ac.be (A. Botteaux), psmeeste@ulb.ac.be (P.R. Smeesters).

https://doi.org/10.1016/j.ijid.2019.01.026

January 2018 in a public tertiary-level hospital located in downtown Brussels. This study was performed to characterize this population and to determine the *emm*-types and *emm*-clusters associated with the GAS strains.

Methods

Study setting

The Brussels-Capital Region has an estimated 1.1 million inhabitants. According to a recent census, around 1200 homeless persons are found in the Region, the majority living on the streets or in public spaces (metro and train stations) of the city of Brussels (Mondelaers, 2017). The Centre Hospitalier Universitaire (CHU) Saint-Pierre is a public hospital and a referral hospital for undocumented or resource-limited patients living in the city of Brussels. The estimated catchment population of the hospital is 122 808 (data provided by the federal Public Service Health, Food Chain Safety and Environment). The hospital is located 1 km from the two main train stations of the region. Every patient admitted to this hospital through the emergency room undergoes a social inquiry interview in which housing and insurance status are collected.

GAS infections in hospitalized patients

From August 2016 to January 2018, all cases of hospitalized adults with an *S. pyogenes* infection confirmed by positive culture identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) were identified prospectively using a laboratory-based alert. Strains isolated from pharyngeal swabs were excluded. Written informed consent was obtained, after which the following data were collected: age, ethnicity, nationality, housing status (homeless or not homeless), alcoholism, use of opioid substitution therapy (methadone or buprenorphine), use of intravenous drugs (IVDU), presence of comorbidities (diabetes, vascular insufficiency, obesity, cancer), steroid use, recent travel history, pregnancy, and HIV, hepatitis B virus (HBV), and hepatitis C virus (HCV) serological testing results. Invasive infections were defined as the isolation of GAS from a sterile site. Strains were frozen for further analysis.

GAS strains in non-hospitalized patients

During the same time period, strains of culture-confirmed *S. pyogenes* (with the exception of strains isolated from pharyngeal swabs) were also collected from non-hospitalized patients. The files of the non-hospitalized patients were retrospectively reviewed for housing status and clinical manifestations.

emm-*type and* emm-*cluster analyses*

GAS identification was confirmed using MALDI-TOF MS, β-hemolysis on blood agar, colony morphology, and positive latex agglutination test (Streptococcal Grouping Kit, Oxoid).

emm-type genotyping was performed as described previously (Centers for Disease Control and Prevention, 2019). *emm*-type interpretation was performed according to the US Centers for Disease Control and Prevention (CDC) classification (Centers for Disease Control and Prevention, 2019) and *emm*-cluster analyses according to the classification published recently by Sanderson-Smith et al. (Sanderson-Smith et al., 2014).

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed by disk diffusion method (Kirby–Bauer) using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (The European Committee on Antimicrobial Susceptibility Testing, 2018).

Statistical analysis

Proportions were compared using the Chi-square test and continuous data were compared by non-parametric Mann–Whitney test. All analyses were performed with R using the Gmisc package (Gordon, Max, 2017; R Core Team, 2017). The Simpson diversity index was calculated using the Vegan package for R (Oksanen et al., 2018) and confidence intervals (95% CI) were calculated as described previously (Grundmann et al., 2001). Incidence rates were estimated as the number of confirmed GAS infections divided by the hospital catchment population, stratified by homelessness status. The homelessness status of the catchment population was not known. Therefore, the most conservative assumption was used, i.e., that all 1200 homeless persons in Brussels were included in the hospital's catchment population.

Results

During the study period, 61 patients with a positive GAS culture from a clinical specimen were hospitalized at CHU Saint-Pierre. Three patients were asymptomatic and were not included in the analyses. The characteristics of the 58 patients with an infection are described in Table 1. The proportion of homeless persons was high (28/58, 48.3%). Considering the catchment area of the hospital and the estimated number of homeless people in the city of Brussels (Mondelaers, 2017), the incidence of GAS infection during the study period was estimated at 2333/100 000 in the homeless group and 25/100 000 in the non-homeless group.

Socio-demographic characteristics and risk factors for GAS infection are shown in Table 1. The majority of patients were male

Table 1
Risk factors for GAS infection among hospitalized patients according to housing status.

	Homeless $n = 28$, n (%)	Non-homeless $n = 30$, n (%)	Total $n = 58$, n (%)	p-Value
Age, mean ± SD	43.9 ± 11.3	43.5 ± 13.2	43.7 ± 12.2	0.91
Sex, male	25 (89.3%)	25 (83.3%)	50 (86.2%)	0.71
Alcoholism	19 (67.9%)	9 (30.0%)	28 (48.3%)	0.008
IVDU	3 (10.7%)	1 (3.3%)	4 (6.9%)	0.34
Methadone OST	10 (35.7%)	3 (10.0%)	13 (22.4%)	0.027
HIV infection				1.0
Negative	25 (89.3%)	25 (83.3%)	50 (86.2%)	
Positive	1 (3.6%)	2 (6.7%)	3 (5.2%)	
Missing	2 (7.1%)	3 (10.0%)	5 (8.6%)	
HCV infection				1.0
Negative	21 (75.0%)	21 (70.0%)	42 (72.4%)	
Positive	5 (17.9%)	6 (20.0%)	11 (19.0%)	
Missing	2 (7.1%)	3 (10.0%)	5 (8.6%)	
HBV infection				1.0
Negative	25 (89.3%)	25 (83.3%)	50 (86.2%)	
Positive	1 (3.6%)	2 (6.7%)	3 (5.2%)	
Missing	2 (7.1%)	3 (10.0%)	5 (8.6%)	
Post-traumatic	16 (57.1%)	10 (33.3%)	26 (44.8%)	0.11
Chronic wound	10 (35.7%)	8 (26.7%)	18 (31.0%)	0.57
Recent travel	0 (0.0%)	3 (10.0%)	3 (5.2%)	0.24
Pregnancy	0 (0.0%)	3 (10.0%)	3 (5.2%)	0.24
Diabetes	0 (0.0%)	5 (16.7%)	5 (8.6%)	0.053
Solid cancer	0 (0.0%)	1 (3.3%)	1 (1.7%)	1.0
Corticosteroid use	1 (3.6%)	1 (3.3%)	2 (3.4%)	1.0
Recent chemotherapy	0 (0.0%)	1 (3.3%)	1 (1.7%)	1.0
Arterial insufficiency	0 (0.0%)	1 (3.3%)	1 (1.7%)	1.0
Venous insufficiency	0 (0.0%)	4 (13.3%)	4 (6.9%)	0.11
Obesity	0 (0.0%)	4 (13.3%)	4 (6.9%)	0.11

GAS, group A *Streptococcus*; SD, standard deviation; IVDU, intravenous drug user; OST, opioid substitute therapy; HCV, hepatitis C virus; HBV, hepatitis B virus.

and the mean age was 43.7 ± 12.2 years. Homeless patients were more frequently users of opioid substitute therapy (35.7% vs. 10%, $p = 0.027$) and suffered more frequently from alcohol abuse (67.9% vs. 30%, $p < 0.01$) than non-homeless patients. A high proportion of post-traumatic infection and chronic wounds were found in both groups. Diabetes was exclusively found in the non-homeless group. Polish nationals were over-represented in the homeless group (28.6% vs. 6.7% in the non-homeless group).

The types of infections observed and clinical outcomes according to housing status are listed in Table 2. Skin infections were predominant in both groups (89.3% in the homeless group and 76.7% in the non-homeless group). There was no difference in the proportion of invasive infection (10.7% in the homeless group vs. 23.3% in the non-homeless group), but homeless persons suffered more frequently from skin abscesses (21.4% vs. 3.3%, $p = 0.048$). The rates of intensive care unit admission and surgery with general anesthesia did not differ between the groups, but mortality was high in the homeless group (10% in the homeless group vs. 3.3% in the non-homeless group). The three deaths observed in the homeless group were Polish nationals with invasive infections (two cases of cellulitis with concomitant bacteremia and one case of necrotizing fasciitis).

Of the GAS strains, 91.4% were susceptible to clindamycin, 84.5% to levofloxacin, and 86.2% to erythromycin. No statistically significant difference in susceptibility was found between the strains from homeless and non-homeless persons.

Twenty-four strains from hospitalized homeless patients and 19 strains from hospitalized non-homeless patients were available for *emm*-typing. The distribution of *emm*-type according to housing status is presented in Table 3. Four *emm*-types (64, 77, 83, and 101) represented 76.1% (16/21) of the *emm*-types in the hospitalized homeless group, while these *emm*-types accounted for 38.8% (7/18) in the non-homeless group ($p = 0.02$). The results of *emm*-cluster analyses are shown in Figure 1A and indicate that, in the hospitalized homeless group, the majority of the *emm*-types belonged to the D4 *emm*-cluster (71.4% vs. 27.8% in the non-homeless group, $p = 0.01$).

Furthermore, 76 non-hospitalized outpatients with a positive *S. pyogenes* culture were identified during the study period (16 homeless and 60 non-homeless). Strains from 15 homeless persons and 51 non-homeless persons were available for *emm*-typing and *emm*-cluster analysis. Skin infections represented the majority of infections in both outpatient groups (15/15 in the homeless group and 38/51 in the non-homeless group). The distributions of the *emm*-types and *emm*-clusters for the outpatients are shown in Table 3. The *emm*-cluster distribution in the outpatient homeless and non-homeless populations are depicted in Figure 1B. The results from *emm*-typing of the outpatients were pooled with the results from inpatients for the analysis of diversity. The Simpson

Table 3
Distribution of *emm*-types among inpatients and outpatients with GAS infection by housing status.

emm-type	*emm*-cluster	Inpatients, *n* (%)		Outpatients, *n* (%)	
		Homeless *n* = 24	Non-homeless *n* = 19	Homeless *n* = 15	Non-homeless *n* = 51
1	A-C3	0 (0)	1 (5.3)	0 (0)	1 (2)
3.93	A-C5			0 (0)	2 (3.9)
4	E1			0 (0)	2 (3.9)
6.58	Clade Y	1 (4.2)	0 (0)		
8	E4			0 (0)	1 (2)
11	E6	0 (0)	1 (5.3)	0 (0)	4 (7.7)
12.37	A-C4		1 (5.3)		
18	Clade Y	0 (0)	1 (5.3)	0 (0)	1 (2)
22	E4	0 (0)	1 (5.3)	0 (0)	0 (0)
27.6	E2	1 (4.2)	0 (0)	2 (13.3)	2 (3.9)
28	E4	0 (0)	1 (5.3)	0 (0)	1 (2)
44	E3			0 (0)	1 (2)
58.17	E3			0 (0)	1 (2)
60.1	E1			0 (0)	1 (2)
64	D4	7 (29.2)	3 (15.8)	1 (6.7)	4 (7.8)
66	E2	1 (4.2)	0 (0)		
75	E6			0 (0)	3 (5.9)
77	E4	2 (8.3)	2 (10.5)	6 (40)	3 (5.9)
81	E6	0 (0)	1 (5.3)	0 (0)	1 (2)
83	D4	4 (16.7)	0 (0)	0 (0)	2 (3.9)
87	E3	0 (0)	1 (5.3)	0 (0)	4 (7.8)
89	E4	0 (0)	1 (5.3)	0 (0)	3 (5.9)
90.2	E2	1 (4.2)	0 (0)		
94.1	E6	0 (0)	1 (5.3)		
95	Outlier			0 (0)	1 (2)
101	D4	3 (12.5)	2 (10.5)	3 (20)	1 (2)
103	E3			0 (0)	1 (2)
108	D4	1 (4.2)	0 (0)	1 (6.7)	0 (0)
109.1	E4			0 (0)	1 (2)
118.2	E3			1 (6.7)	1 (2)
165	E1			0 (0)	1 (2)
209	E3	0 (0)	1 (5.3)		
223	D4			0 (0)	1 (2)
STG652				0 (0)	1 (2)
NT		3 (12.5)	1 (5.3)	1 (6.7)	6 (11.8)

GAS, group A *Streptococcus*; NT, non-typeable.

Table 2
Infection type and outcome according to homeless status among hospitalized patients with a GAS infection.

	Homeless *n* = 28, *n* (%)	Non-homeless *n* = 30, *n* (%)	Total *n* = 58, *n* (%)	*p*-Value
Type of infection				
Cellulitis	13 (46.4%)	14 (46.7%)	27 (46.6%)	1.0
Invasive infection	3 (10.7%)	7 (23.3%)	10 (17.2%)	0.3
Skin abscess	6 (21.4%)	1 (3.3%)	7 (12.1%)	0.048
Skin ulcer	1 (3.6%)	3 (10%)	4 (6.9%)	0.6
Postoperative infection	0 (0%)	2 (6.7%)	2 (3.4%)	0.49
Impetigo	2 (7.1%)	0 (0%)	2 (3.4%)	0.23
Mastoiditis	1 (3.6%)	0 (0%)	1 (1.7%)	0.48
Chorioamnionitis	0 (0%)	1 (3.3%)	1 (1.7%)	1.0
Osteitis	1 (3.6%)	0 (0%)	1 (1.7%)	0.48
Otitis	1 (3.6%)	0 (0%)	1 (1.7%)	0.48
Tonsillar abscess	0 (0%)	1 (3.3%)	1 (1.7%)	1.0
Vaginal infection	0 (0%)	1 (3.3%)	1 (1.7%)	1.0
Outcome				
ICU admission	4 (14.3%)	4 (13.3%)	8 (13.8%)	1.0
Surgery with general anesthesia	6 (21.4%)	5 (16.7%)	11 (19%)	0.74
Death	3 (10.7%)	1 (3.3%)	4 (6.9%)	0.34

GAS, group A *Streptococcus*; ICU, intensive care unit.

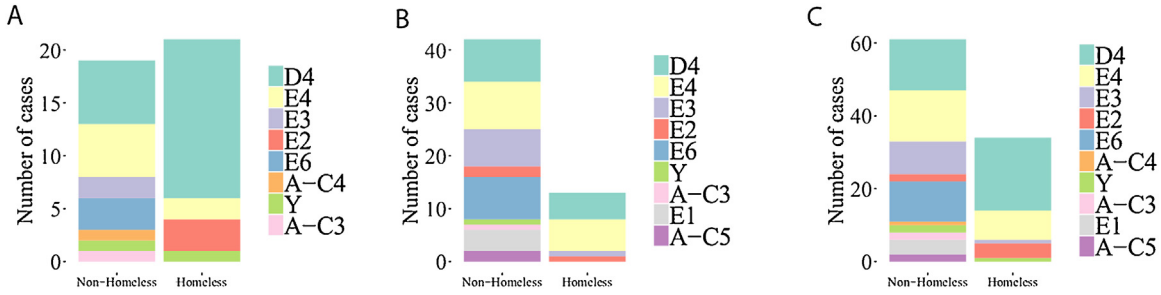

Figure 1. Distribution of *emm*-clusters by housing status among (A) inpatients, (B) outpatients, and (C) both inpatients and outpatients.

diversity index was significantly lower in the homeless group: 0.84 (95% CI 0.79–0.89) vs. 0.95 (95% CI 0.93–0.96) in the non-homeless group. There was a significant difference in the distribution of *emm*-clusters between the homeless and non-homeless groups when considering both inpatient and outpatient strains ($p < 0.001$, Chi-square test) Figure 1C.

Discussion

This study reports a high proportion of homeless persons among adults hospitalized with a GAS infection at a tertiary-care hospital located in the city of Brussels, the municipality where the majority of homeless persons were identified in the Brussels-Capital Region during a recent census (Mondelaers, 2017).

Based on the catchment area of the hospital and the estimated population of homeless people in Brussels City, the incidence rate during the study period was 100 times higher in the homeless population. Previous GAS outbreaks in homeless populations described in Europe and North America have predominantly reported high proportions of invasive infections caused by single *emm*-type strains (Cady et al., 2011; Bundle et al., 2017; Mosites et al., 2018). In the present study setting, a majority of skin infections was observed, with a high proportion of abscesses. There were only three cases of invasive infection reported in the homeless group, but all three patients died. A limited *emm*-type distribution was found in the homeless population and four *emm*-types were over-represented (64, 77, 83, and 101). The majority of *emm*-types found in the hospitalized homeless population belonged to the D4 *emm*-cluster. This cluster has previously been associated with skin infections (Sanderson-Smith et al., 2014). D4 *emm*-cluster GAS strains have the ability to bind plasminogen. Interestingly, plasminogen has been shown to accumulate in wounds during the healing process, in which it plays a critical role (Shen et al., 2012). An important proportion of homeless inpatients suffered from chronic and post-traumatic wounds.

Prevention messages were transmitted to the task force responsible for homeless care in Brussels. These preventive messages included the need to attend care in the case of wounds and the promotion of hygiene, and special attention was paid to the Polish subgroup. Mass administration of antibiotics was used in a recent prolonged outbreak of invasive GAS disease in the homeless population of Anchorage, Alaska (Mosites et al., 2018). In this setting, the homeless people were localized in a limited number of service facilities. This strategy was not considered in Brussels, due to the low rate of invasive infection and the high proportion of homeless patients living on the streets.

Caution must be taken when generalizing these results because of the limited size of the study and the limited number of strains that were characterized by *emm*-typing. Moreover, the estimation of the incidence rates relied on assumptions concerning the catchment populations for both groups, possibly introducing bias. However, the limited number of *emm*-types in both inpatients and outpatients suggests endemic circulation of selected GAS strains in this population. As well as the preventive strategy based on access to care and enhanced hygiene as stated above, immunization, when available, would likely represent an efficient tool to decrease the burden of this infection in this fragile population.

Acknowledgements

We thank Gilles Dauby PhD for his expertise in the R analyses.

Funding

This work was supported by F.R.S.-FNRS research grants (PDR T.0255.16 and CDR J.0019.17) and the Fund Iris-Research (managed by the King Baudouin Foundation). N.D. is a post-doctorate clinical master specialist of the F.R.S.-FNRS.

Ethical approval

The study was approved by the Ethics Committee of CHU Saint-Pierre. Informed consent was provided by the hospitalized subjects.

Conflict of interest

No conflict of interest to declare.

References

Bundle N, Bubba L, Coelho J, Kwiatkowska R, Cloke R, King S, et al. Ongoing outbreak of invasive and non-invasive disease due to group A Streptococcus (GAS) type emm66 among homeless and people who inject drugs in England and Wales, January to December 2016. Euro Surveill 2017;22(January (3)).

Cady A, Plainvert C, Donnio P-Y, Loury P, Huguenet D, Briand A, et al. Clonal spread of *Streptococcus pyogenes* emm 44 among Homeless persons, Rennes, France. Emerg Infect Dis 2011;17(February (2)):315–7.

Centers for Disease Control and Prevention. Streptococcus laboratory. Blast-emm & emm Databases. 2019 [cited 2019 January 4]. Available from: https://www2a.cdc.gov/ncidod/biotech/strepblast.asp.

Gordon, Max. Gmisc: descriptive statistics, transition plots, and more. 2017 Available from: https://CRAN.R-project.org/package=Gmisc.

Grundmann H, Hori S, Tanner G. Determining confidence intervals when measuring genetic diversity and the discriminatory abilities of typing methods for microorganisms. J Clin Microbiol 2001;39(November (11)):4190–2.

Mondelaers N. Dénombrement des personnes sans abri et mal logées en Région de Bruxelles-Capitale. Bruxelles: La STRADA; 2017.

Mosites E, Frick A, Gounder P, Castrodale L, Li Y, Rudolph K, et al. Outbreak of invasive infections from subtype emm26.3 Group A streptococcus among homeless adults—Anchorage, Alaska, 2016–2017. Clin Infect Dis 2018;66(March (7)):1068–74.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. vegan: community ecology package. 2018 Available from: https://CRAN.R-project.org/package=vegan.

R Core Team. R. A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2017 Available from: http://www.r-project.org/.

Sanderson-Smith M, De Oliveira DMP, Guglielmini J, McMillan DJ, Vu T, Holien JK, et al. A systematic and functional classification of *Streptococcus pyogenes* that

serves as a new tool for molecular typing and vaccine development. J Infect Dis 2014;210(October (8)):1325–38.
Shen Y, Guo Y, Mikus P, Sulniute R, Wilczynska M, Ny T, et al. Plasminogen is a key proinflammatory regulator that accelerates the healing of acute and diabetic wounds. Blood 2012;119(June (24)):5879–87.
The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, version 8.0. 2018 Available from: http://www.eucast.org/clinical_breakpoints/.