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# Human leptospirosis cases in Palermo Italy. The role of rodents and climate

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### ABSTRACT

Many regions of the world are increasingly exposed to leptospirosis due to poverty, global warming and high urban density. Here, we report a molecular survey for pathogenic *Leptospira* spp. in rodents and two symptomatic human cases of leptospirosis in the city of Palermo, Italy.

Four rodent species were captured in six areas of the city, and a molecular analysis for pathogenic *Leptospira* spp. on DNA from the kidney samples showed a different prevalence of leptospirosis in all the species of rodents. In addition, two human cases that occurred in May and October of 2009 in the city were also reported. A 67-year-old woman recovered after antibiotic treatment, whereas a 71-year-old woman did not survive. The weather during both of those times was notable for a violent cloudburst that caused street flooding.

For the past several years, the incidence of leptospirosis has remained steady at 9 human cases every 10 years across the entire island of Sicily, with a population of almost 5 million inhabitants.

The high prevalence of leptospirosis in rodents and the simultaneous presence of known risk factors, such as a mild/wet climate, street flooding and garbage accumulation, could explain the two cases of leptospirosis within the same city in the same year. This occurrence should raise awareness of this underestimated zoonosis among public health authorities, especially given the potential fatality among elderly and immune-compromised individuals in urban settings in developed countries.

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### Introduction

Leptospirosis is one of the most widespread bacterial zoonoses in the world. It is caused by over 200 different serovars belonging to several serogroups of the genus *Leptospira* [1]. Clinical presentation ranges from mild, flu-like symptoms to severe symptomatology, including Weil's syndrome, with multi-organ failure and, often, fatal pulmonary hemorrhagic syndrome [2]. Contact with stray animals, rodents, poor sanitation, heavy rainfall and flooding are the main risk factors in developing countries, whereas recreational activities, such as freshwater swimming, fishing, or sporting events are associated with clinical leptospirosis in developed countries [3,4]. Many regions of the world are increasingly exposed to leptospirosis infection and disease due to climate change, global

warming, a high urban density and poverty. Developing countries carry the major burden of the disease, with half a million cases reported yearly and a mortality rate ranging from 5 to 10% [5]. However, global warming also impacts leptospirosis cases in developed countries. In the Netherlands, an overall decreasing trend of leptospirosis over the past decades has recently reverted to a marked increase in cases among humans and dogs [6,7]. Rodents are the main animal reservoir in urban settings, with *Rattus norvegicus* primarily involved in pathogenic *Leptospira interrogans* serovar Copenhageni transmission [8]. Leptospirosis is reported at a high prevalence in the rodent population of major cities in developed countries, such as Baltimore in the USA [9], Tokyo in Japan [10] and Copenhagen in Denmark [11]. In Italy, the majority of cases are usually recorded in the northern regions of Lombardy, Piedmont and Veneto, where a decrease in the annual incidence of human leptospirosis has been recorded since 1996 [12]. Sporadic cases of symptomatic leptospirosis have been reported in these northern Italian regions, and they are often related to river flooding [13].

In this work, two human cases of leptospirosis that occurred in spring and fall in 2009 in the city of Palermo are discussed together

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with the results of a molecular survey of rodents captured in green and residential areas of the same city in 2008–2009 for pathogenic *Leptospira* spp. Usually, only nine cases of clinical leptospirosis in ten years are reported across Sicily (Regional Reference Centre, Vitale G.), while in 2009, a serious case and a fatal case in Palermo were recorded in the same year.

## Materials and methods

### Human leptospirosis cases

In 2009, two clinical leptospirosis cases were reported in the city of Palermo during the spring and fall seasons. Leptospirosis was confirmed using a microscopic agglutination test (MAT) on the patient's serum according to the WHO guidelines [14] and with Elisa Pambio IgM as previously described [15].

### Rodent captures

Based on the number of rodent control interventions, 22 locations were monitored in residential and green areas. The locations were grouped in 6 larger areas (1–6 in Fig. 1), and Havahart traps (for rats) and Longworth traps (for *Apodemus sylvaticus* and *Mus domesticus* species) were used, as previously described [16], to capture the rodents. The rodents were euthanized by a 5-min inhalation of CO<sub>2</sub> followed by a bilateral thoracotomy. Samples from both kidneys (2 g) were used for DNA extraction. A total of

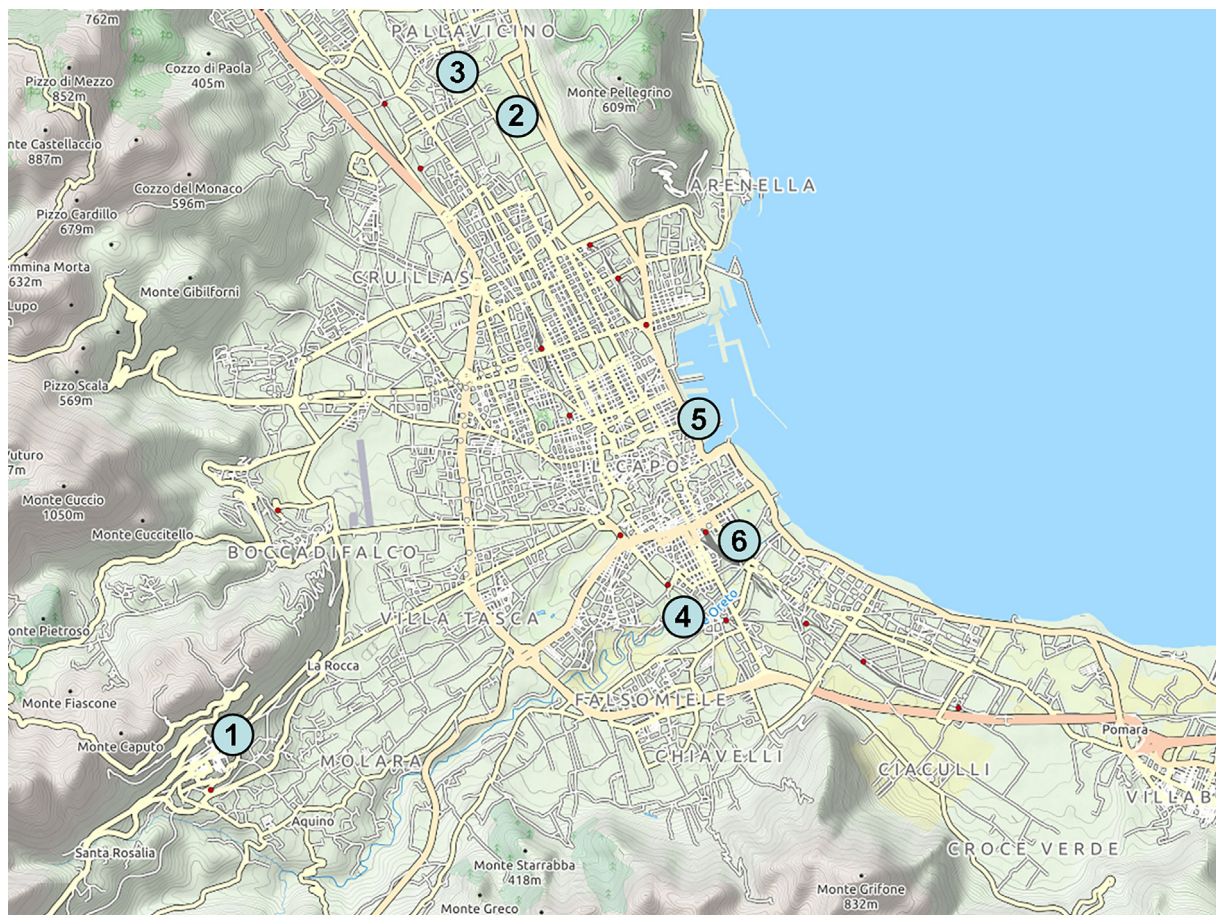
243 rodents, including 27 that had been randomly killed by local residents, were analyzed.

### Molecular analysis of the rodents

Rodent kidney tissues were dissolved in 2 ml of 10 mM TRIS-1 mM EDTA pH 8 and were homogenized using an EDTS VIII homogenizer (Design Village Ltd, U.K.). Total genomic DNA was extracted from the homogenates using a Gene Elute mammalian kit (cat N° G1N350 Sigma-Aldrich St. Louis, MO, USA) following the manufacturer's instructions. A PCR analysis the 16S rRNA gene was conducted as previously described [17]. The primers used to test for pathogenic *Leptospira* spp. were LEPTO E1 GGGAAAAATAAGCAGCGATGTG (forward) and LEPTO E2 (reverse) ATTCCACTCCATGTCAAGCC. The following program, in the Applied Biosystems 9700 thermal cycler, was used: 94 °C for 5 min; 1 cycle followed by 40 cycles at 1 min at 94 °C, 1 min at 60 °C, 1 min at 72 °C; and the final extension step at 72 °C for 5 min.

### Sequencing analysis

Thirty positive amplicons were also confirmed by a sequencing analysis of the amplified fragments. The reactions were carried out with the primers LEPTO E1 and LEPTO E2 using the Big Dye Terminator Cycle sequencing Kit v3.1 (10'' at 96 °C, 5'' at 60 °C, 4' at 60 °C for 25 cycles) and were detected using an ABI PRISM 3130 appara-



**Fig. 1.** Map of the city of Palermo and the main areas of rodents captures.

A schematic map of the city of Palermo in which the 6 main areas where the rodents were captured are shown by numbered circles. (1) Base of Monreale Hill (38.081998, 13.291698); (2) Ippomontato (Favorita Park) (38.161786, 13.337624); (3) Rom Camp (Favorita Park) (38.159367, 13.336788); (4) Oreto river (38.098184, 13.362194); (5) Fish Market (38.121169, 13.366215); (6) Municipal Kennel (38.107053, 13.373096). The captures were planned in collaboration with Ecological service of Palermo.

**Table 1**  
results by species of the total 243 rodents analyzed by PCR.

|              | <i>Rattus norvegicus</i> | <i>Mus domesticus</i> | <i>Rattus rattus</i> | <i>Apodemus sylvaticus</i> |
|--------------|--------------------------|-----------------------|----------------------|----------------------------|
| Total        | 114                      | 85                    | 31                   | 13                         |
| PCR positive | 41                       | 33                    | 7                    | 2                          |
| %            | 35,96                    | 38,82                 | 22,58                | 15,38                      |
| 95% CI       | 27.7–45.1                | 29.1–49.4             | 11.1–40.1            | 3.1–43.5                   |

A total of 243 rodents were analyzed by a PCR specific to pathogenic *Leptospira* spp. Four species of rodents were captured, including *Rattus norvegicus*, *Mus domesticus*, *Rattus rattus*, and *Apodemus sylvaticus*. All species presented positive specimens at different percentages. CI, confidence interval in italics. Italics are the values of Confidence interval In bold are the values referred to the Oreto river area which is related to the fatal case.

tus (Applied Biosystems). The sequence alignment was carried out using Web BLAST (NCBI).

### Statistical analysis

The X2 (chi-square) test was used to determine significant differences in the prevalence of *Leptospira* spp. Among the host species from the various capture areas. The confidence intervals were calculated using the modified Wald method [18] with GraphPad Software online.

### Theory

Rodents are the main animal reservoir for leptospirosis in urban settings. In this paper, we postulated that the rare occurrence of two clinical cases of leptospirosis in 2009 in the city of Palermo was probably related to heavy rain episodes that caused flooding in urban streets and riverbanks. During the street floods, many individuals were potentially in contact with water contaminated by infected rodent urine, increasing their risk of infection. However, because of the good hygienic conditions and economic wellness in developed countries, the appearance of severe symptoms is usually very rare. It is also possible that periodic exposures to the locally circulating serovars render the immune-competent population more resistant to the infection so that only few individuals (elder or immune-compromised) develop the clinical signs. In the city of Vicenza in Northern Italy, for example, one fatal case in an elderly individual and elevated sero-conversion rates in the general population were reported after a river flooding [18].

## Results

### Human cases

**Case 1.** A 67-year-old housewife who lived in the city of Palermo, was admitted at the hospital in May 2009. Her symptoms included jaundice, hepatomegaly, splenomegaly with hyperbilirunemia, neutrophilic leukocytosis and progressive renal failure with increased levels of blood urea nitrogen, creatinine and creatine kinase. MAT analyses on the sera were carried out three times (on

the day of admission and 7 days and 20 days afterwards). At the time of hospital admission, the analysis of acute serum showed a positive reaction to the *L. interrogans* serovar Hardjo strain Hardjoprajitno, with a titer of 1:320. After 7 days, the following results were obtained: *L. interrogans* serovar Hardjo (1:320) and *L. interrogans* serovar Icterohaemorrhagiae (1:100). After 20 days, *L. interrogans* serovar Icterohaemorrhagiae, with a titer of 1:3200, was detected. The patient recovered after an intravenous antibiotic treatment. The patient confirmed that almost two weeks prior to admission she was involved in an urban street flood with her car during heavy rain (Vitale G. personal communication).

**Case 2.** A 71-year-old woman who lived in the outskirts of Palermo in the area of the river Oreto was admitted at the hospital in October 2009 with fever, general malaise, rubella-like exanthema and atrial fibrillation. Her clinical course showed fever, jaundice, hepato-splenomegaly, kidney failure, and meningitis. On the first day of admission, anemia and thrombocytopenia were found with increased reactive protein C levels and altered liver and kidney function tests. Urine testing showed hematuria, proteinuria and bacteriuria. Positive results were obtained by MAT for *L. interrogans* serovar Hardjo strain Hardjoprajitno 1:400. The PanBio ELISA IgM analysis was also positive. The woman related that she had manipulated greens cultivated along the Oreto riverbank. Once leptospirosis was confirmed, the appropriate antibiotic was initiated, but she died within 24 h of the therapy. Her clinical history was recently exhaustively reported elsewhere [19].

### Molecular survey in the rodent population

In a total of 243 rodents, 114 (53.6%) were *R. norvegicus*, 31 were (14.7%) *Rattus rattus*, 85 (29%) *M. domesticus* and 13 (4.6%) *A. sylvaticus*, a small mouse living in the woodlands and peri-urban areas. A large urban green area of the city is represented by La Favorita park, (number 2 in Fig. 1), which is the only place in which all four species of rodents were simultaneously present. Most of the captured animals were adults, with a ratio of 1:1 male/female. *Leptospira* spp. positive animals were found in individuals of all species with a prevalence ranging from almost 39% in the commensal species *M. domesticus* to 15% in *A. sylvaticus* (Table 1). A higher density of general rodent population was observed in peri-urban green areas

**Table 2**  
Rodent samples captured in different Palermo urban areas.

|              | Rodents in green areas |           |           |                  | Rodents in residential areas |          |           | Total     |
|--------------|------------------------|-----------|-----------|------------------|------------------------------|----------|-----------|-----------|
|              | (1)                    | (2)       | (3)       | (4) Oreto River  | (5)                          | (6)      | *(7)      |           |
| Total sample | 19                     | 124       | 31        | <b>17</b>        | 13                           | 12       | 27        | 243       |
| PCR positive | 7                      | 42        | 8         | <b>9</b>         | 2                            | 2        | 11        | 81        |
| Prevalence % | 36,8                   | 33,9      | 25,8      | <b>52,9</b>      | 15,4                         | 16,6     | 40,7      | 33,3      |
| 95% CI       | 19.1–59.1              | 26.1–42.6 | 13.5–43.5 | <b>30.9–73.8</b> | 3.1–34.5                     | 3.5–46.0 | 24.5–59.3 | 27.7–39.5 |

A total of 243 rodents from different locations (1–7) were analyzed by a PCR specific to pathogenic *Leptospira*. \*(7) Individual occasionally killed rodents. CI, confidence interval. In bold, the results refer to area 4, the Oreto river estuary The area in which the woman of the fatal leptospirosis case lived. The numbered areas correspond to the spots in the schematic map of Fig. 1: (1) Base of Monreale Hill (38.081998, 13.291698); (2) Ippomontato (Favorita Park) (38.161786, 13.337624); (3) Rom Camp (Favorita Park) (38.159367, 13.336788); (4) Oreto river (38.098184, 13.362194); (5) Fish Market (38.121169, 13.366215); (6) Municipal Kennel (38.107053, 13.373096). Italics are the values of Confidence interval In bold are the values referred to the Oreto river area which is related to the fatal case.



compared to the residential areas. This is probably due to a minor intervention for the control of the rodent population in these areas. A schematic map of the city with the six hot spot areas is shown in Fig. 1. The data of the molecular analysis, on the randomly collected dead rodents versus the trapped and captured animals, are described in Table 2. The captured rodents were grouped according to the origin of the samples (green and residential areas); the locations were numbered 1–6 and corresponded to those in Fig. 1. The highest prevalence of leptospirosis (52.9%) was detected in area 4 (bold in Table 2), corresponding to the Oreto river estuary in which mainly *R. norvegicus* and *R. rattus* were present. No statistically significant difference was found in the prevalence of *Leptospira* spp. among the host species and the areas of capture.

### Sequencing results

The thirty positive amplicons, randomly chosen for sequencing from the different rodent species, showed >98% sequence homology to the *L. interrogans* 16S rRNA gene [GenBank X14249 Web 1].

### Social welfare and climate in Palermo

The reduction of social welfare support related to the 2008–2009 economic crisis was evident in Sicily and in Palermo and combined with a high unemployment rate [Web 2–3]. In the Köppen's classification system of the climate, Sicily is considered a zone 'CSA' with a temperate climate ('C'), dry summers ('S'), and an average temperature above 22 °C ('A') in the warmer months. However, the climate is changing with an increase in average temperature and rain volume [Web 4].

In April 2009, frequent storms occurred in different areas of the region, including Palermo (particularly on April 20th, 17 days before the first case) [Web 5].

The woman involved in the first case reported that nearly 20 days earlier she had been on a flooded road during a violent thunderstorm, because her car was blocked due to a wet engine.

In September of the same year, there were three heavy rainfall episodes (on the 16th, 21st and 25th) [Web 6]. During these heavy rains, floods with potentially contaminated water occurred in urban streets. In addition, the water level of the Oreto river increased, and numerous gardens along its shores flooded (the second case occurred on October 13th).

## Discussion

### Leptospirosis in rodents

Molecular testing for leptospirosis showed that positive specimens were present in all the species of wild rodents with a percentage ranging from 38% in *M. domesticus* to 15% in *A. sylvaticus* (Table 1). A high prevalence was present in *M. domesticus* and *R. Norvegicus* both living in almost of the areas of the city. *M. domesticus* is the main rodent responsible for leptospirosis transmission on the Island of Terceira in the Azores archipelagos [20], whereas *R. norvegicus* is involved in the human transmission in Madagascar [21]. A high prevalence of pathogenic *Leptospira* in *M. domesticus* and *R. rattus* was also described for the island of Cape Verde, highlighting a serious health risk for the local population [22]. Mice and rats are natural reservoirs for pathogenic host-related *Leptospira* serovars with *M. domesticus* being the natural host for the serovars Ballum and Arborea and *R. norvegicus* for Icterohaemorrhagiae, Copenhageni and Lai [23].

### Human cases

A high titer for *L. interrogans* serovar Icterohaemorrhagiae was detected in the third serum sample of the first human case we described (20 days after hospitalization). The patient was in contact with potentially contaminated water in a street flood after a violent cloudburst in the previous month. In addition, the leptospirosis fatal case occurred in a woman with a late diagnosis of leptospirosis due to an incorrect interpretation of a rash as a drug-reaction and not a vasculitis-like reaction [19]. Her history did not relate any contact with animals, any travel or any swimming in fresh water, but she had manipulated vegetables cultivated along the Oreto riverbank, where several urban orchards are present. Although a direct relation cannot be demonstrated, the potential risk for leptospirosis transmission through urban gardening is reported in arid countries [24]. Similar to the first case, the MAT analysis at admission was positive for the *L. interrogans* serovar Hardjo strain Hardjopratio at 1:400. This serovar is usually associated with cattle leptospirosis and along the banks of the Oreto River there are also grazing cattle sometimes, but since no further samples from the patient were taken this data could not be confirmed. However, it is possible that the result showing *L. interrogans* serovar Hardjo in the acute serum sample was due to the "paradoxical reactions", in the early phases of the disease when heterologous reactions are stronger than the homologous reactions [25]. The Oreto "river" currently shows a low volume, which is similar to that of a creek, and in this study, a very high prevalence of positive rodents (52%) mainly *R. norvegicus* were found in its estuary (Table 2 area 4 in bold). Previously, similar results were reported along the banks of the Tiber river in Rome [26], a city that shares many common problems with Palermo and other large cities in South Italy, such as urban street floods from heavy rains and waste accumulation. A further risk factor for leptospirosis, in the city of Palermo, is represented by a population of almost 10 thousand stray dogs feeding on garbage containers with the molecular prevalence for leptospirosis ranging from 15 to 18% [27].

### Waste accumulation

The waste collection problem in Palermo likely facilitates the increased rodent population as suggested by an increasing number of requests for rodent control interventions to City Hall (source: local ecological office). A direct correlation between garbage accumulation in the streets and human leptospirosis cases is reported in the city of Marseille, where strikes by the operators for waste collection are related with the increasing presence of rodents and the report of autochthon cases of human leptospirosis [28].

### Leptospirosis epidemiology

In Sicily, a yearly incidence of less than one case of human leptospirosis in the whole island is usually reported, and an average of 30 serological tests for *Leptospira* spp. are performed each year upon clinical suspicion or incidental contacts with potentially infected animals (The Sicilian regional reference laboratory for tropical infectious disease, G. Vitale). A higher incidence of leptospirosis is more common in the Northern regions of Italy, and the area surrounding the city of Vicenza was characterized by endemic leptospirosis until the 1980s with a decreasing trend over the last few decades. However, a fatal case of hemorrhagic pneumonia in an elderly man and a sero-conversion rate of 6.8% in the local population were detected in Vicenza city after a significant flood [18].

It is possible that, like vaccinations, serum conversion protects the healthy population against circulating serotypes of pathogenic *Leptospira*, but when health conditions deteriorate or there are contacts with unusual serovars, symptomatic leptospirosis may

appear. In the city of Venice, two cases of severe leptospirosis were observed, in two young and healthy Australian tourists, after they dove in the lagoon, an illegal practice that local citizens do not perform [29]. In this particular city, occasional contact with potentially contaminated water can occur several times throughout the year, but a higher incidence of human leptospirosis in Venice has never been detected. However, general screening on the population has never been conducted to date and to address this potential health issue, a serology screening in Venetians might provide useful information. A serological screening in Austria in a normal male population showed a seroprevalence of 23% for *Leptospira* spp. [30].

### *Leptospira* and poverty

Leptospirosis is largely associated with poverty and great epidemics of severe leptospirosis are reported in urban slums in developing countries [31], where the impact of socioeconomic factors and the environmental gradient on leptospirosis infections is evaluated [32]. In developed countries, careful health and hygiene practices, attention to the cleanliness of urban areas and the use of antibiotic treatments at early febrile symptoms help to ensure the control of the symptomatic disease in immune-competent individuals. However, leptospirosis is also considered a neglected infection associated with poverty in non-European developed countries, such as the United States [33] and Japan [10].

The reduction of social welfare is evident in Palermo and Sicily, with a high number of unemployed people, particularly among the young generation [Web 3]. The leptospirosis cases in 2009 involved two housewives with a lower-middle class income, but no other severe cases were recorded in Palermo thus far. However, considering that only symptomatic severe leptospirosis cases are referred to hospitals, information about the true prevalence of infection is probably underestimated.

### Conclusion

The high prevalence of leptospirosis in rodents and the simultaneous presence of risk factors, such as a mild/wet climate, the flooding of urban streets, and socio-economic problems, could explain the two cases of severe human leptospirosis found in the same city in 2009.

Other Italian cities carry the presence of simultaneous risk factors for leptospirosis, and thus, a major concern from this underestimated zoonosis should be considered by public health authorities and clinicians especially for elderly and immune-compromised individuals.

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### Competing interests

None declared.

### Ethical approvals

The rodents were captured in collaboration with the department of ecology of Palermo, which normally performs procedures for rodent control. Local regulations regarding animal welfare were observed in relation to the Italian Legislative Decree 116/92, Directive 86/609/EE European Union in accordance with the annexed guidelines. In addition, individual rodents killed occasionally by residents in different areas of the city were included in the analysis.

The data of the two human clinical cases reported here (with their anamneses) were obtained from the reports of the “Regional center of infectious diseases” (Palermo, Italy). The study was conducted according to the ethical guidelines from the declaration of Helsinki and approved by the local ethical committee (Ref IRB Polyclinic Hospital, Palermo, Italy). Informal written consent from the patients for the use of their information for research purposes and database collection was given at the moment of Hospital admission.

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