International Journal of Infectious Diseases 154 (2025) 107843

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

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

The increasing prevalence of Enterobacteriaceae as pathogens of diabetic foot osteomyelitis: A multicentre European cohort over two decades

Laura Soldevila-Boixader[1,2,3,4], Oscar Murillo[3,5,*], Felix W.A. Waibel[6], Madlaina Schöni[6], Javier Aragón-Sánchez[7], Karim Gariani[8], Dan Lebowitz[9], Bülent Ertuğrul[10,11], Benjamin A. Lipsky[12], Ilker Uçkay[1,2,6]

[1] Department of Infectiology, Balgrist University Hospital, Zurich, Switzerland
[2] Unit for Clinical and Applied Research, Balgrist University Hospital, University of Zurich, Zurich, Switzerland
[3] Department of Infectious Diseases, Bellvitge University Hospital, L'Hospitalet de Llobregat, Barcelona, Spain
[4] Internal Medicine, Infectious Diseases Unit, Complex Hospitalari Moisès Broggi, Consorci Sanitari Integral, Sant Joan Despí, Barcelona, Spain
[5] CIBERINFEC (Centro de Investigación Biomédica en Red en Enfermedades Infecciosas), Instituto de Salud Carlos III, Madrid, Spain
[6] Department of Orthopedic Surgery, Balgrist University Hospital, Zurich, Switzerland
[7] Department of Surgery and Diabetic Foot Unit, La Paloma Hospital, Las Palmas de Gran Canaria, Spain
[8] Division of Endocrinology, Diabetes, Nutrition and Therapeutic Patient Education, Geneva University Hospitals, Geneva, Switzerland
[9] Service of Infectious Diseases, Geneva University Hospitals, Geneva, Switzerland
[10] Unit for Diabetic Foot and Chronic Wounds, Service of Infectious Diseases and Clinical Microbiology, GEDA Clinic, Izmir, Turkiye
[11] Service of Infectious Diseases and Clinical Microbiology, REYAP Clinic, Istanbul, Turkiye
[12] Department of Medicine, University of Washington, Seattle, Washington, USA

ARTICLE INFO

Article history:
Received 7 October 2024
Revised 8 February 2025
Accepted 10 February 2025

Keywords:
Diabetic foot osteomyelitis
Multicentre cohort
Gram-negative rods
Enterobacteriaceae
MRSA

ABSTRACT

Objectives: To investigate the microbiological trends of community-acquired diabetic foot osteomyelitis (DFO) over the past two decades in specialized academic centres in Switzerland, Spain, and Turkey.
Methods: A retrospective analysis of DFO cohorts (2000-2019) from five centres (Geneva, Zurich, Las Palmas, Barcelona, Istanbul) stratified into four periods (P1-P4) to assess microbiological changes.
Results: Among 1379 DFO episodes (76% male, median age 67 years; 90% type 2 diabetes, median duration 17 years), gram-positive bacteria were identified in 82%, including *Staphylococcus aureus* (47%). Methicillin-resistant *S. aureus* (MRSA) was more prevalent in Barcelona (36%), Las Palmas (24%), and Geneva (29%) than in Zurich (7%). Over time, gram-positive bacteria remained stable or decreased, particularly in Las Palmas (83% to 65%, $P = 0.03$). The proportion of MRSA decreased in Geneva (39% to 16%) and Las Palmas (37% to 9%), but remained stable in Barcelona. Enterobacteriaceae prevalence increased, notably in Geneva (16% to 39%, $P < 0.01$) and Las Palmas (27% to 41%, $P < 0.01$). Among gram-negative pathogens quinolone resistance was 12.5%. Enterobacteriaceae-DFO was associated with ischemic necrosis (OR 1.65), Las Palmas cohort (OR 3.14), and 2016-2019 period (OR 2.68).
Conclusions: A significant increase in Enterobacteriaceae-related DFOs was observed from 2016 to 2019, particularly in Mediterranean Europe.

Introduction

The management of diabetic foot osteomyelitis (DFO) requires a multidisciplinary approach, including pressure off-loading, glycaemic control, microbiological assessment, surgical intervention, and culture-based antibiotic therapy [1–5]. The causative pathogens are often polymicrobial and influenced by patient characteristics, previous antibiotic therapy, orthopaedic implants [6] and geographic associated epidemiology.

In the northern hemisphere (predominantly resource-rich countries), methicillin-susceptible *Staphylococcus aureus* has been the most commonly isolated DFO pathogen, followed by other aerobic gram-positive bacteria such as coagulase-negative staphylococci

* Corresponding author. Oscar Murillo, Department of Infectious Diseases, Bellvitge University Hospital Hospitalet de Llobregat, Carrer de la Feixa Llarga, s/n, 08907 L'Hospitalet de Llobregat, Barcelona, Spain
E-mail address: omurillo@bellvitgehospital.cat (O. Murillo).

https://doi.org/10.1016/j.ijid.2025.107843

and skin commensals [7], gram-negative rods (e.g. *Pseudomonas* spp., Enterobacteriaceae) [8] or gram-negative anaerobes [9]. In contrast, in resource-poor countries, and especially in South Asia and semi-arid (sub)tropical regions, gram-negative bacteria frequently dominate over staphylococci in diabetic foot infections (DFI), for as yet incompletely explained reasons [10,11].

This global difference in DFI microbiology may be favouring gram-negatives. Over the past fifteen years, there has been a worldwide increase in infections caused by gram-negative bacilli [12], including in osteoarticular infections [13] like DFO [14]. Infections caused by the Enterobacteriaceae family are of particular concern. This is because of their great numbers in the community, with frequent spillover into the hospitals, and their raising rates of antibiotic resistance to most first-line agents [10,12,15–17], including quinolones (the main oral therapy). This shift may have serious adverse consequences for both patients and health care systems, since infections caused by gram-negative infections, with high rates of antibiotic (multi)-resistance [10–17] compared to gram-positive pathogens, are associated with more prolonged inflammation [18], higher rates of limb loss [19], more frequent antibiotic overuse [20] and higher mortality in severe DFI [2].

For the present study, we shared the information from several European countries to compile a large database for research purposes. Our main objective was to investigate if there were any microbiological changes in the causative pathogens of DFO over the study time and by the geographic location. We specifically focused on DFO caused by Enterobacteriaceae, investigating their trends and seeking to identify any variables that might be associated with this etiology.

Material and methods

Study criteria

We focused on community-acquired DFOs in adult patients who individually consented for their data to be used for research purposes, with a minimum follow-up of six months after treatment. We defined DFO according to guidelines formulated by the Infectious Diseases Society of America (IDSA) [2] or International Working Group on the Diabetic Foot (IWGDF) [4]. After review, we excluded what we considered implausible cases from the analysis (doubtful diagnosis or incomplete data, implant-related DFO episodes, and exclusively soft-tissue or nosocomial infections). A detailed flowchart outlining the inclusion and exclusion criteria can be found in Supplementary Figure 1.

We used only microbiological assessments of DFO from bone biopsies, ensuring a higher accuracy of the results. We used the outcome "Enterobacteriaceae" to represent either a monobacterial infection or a dominant pathogen in a polymicrobial DFO.

Research group and composite database

We used a composite database of five cohorts of patients diagnosed with community-acquired DFI over the last two decades (from 1 January 2000 to 31 December 2019) in three European countries: Zurich and Geneva in Switzerland; Barcelona and Las Palmas de Gran Canaria in Spain; and Istanbul in Turkey. These cities represent the longitudinally opposite locations in Switzerland and Spain, with Barcelona and Istanbul as opposites in "Mediterranean Europe," while the Swiss centres serve as a "control" in Central Europe.

The database study periods were as follows: Zurich 2000-2019; Geneva 2000-2019; Las Palmas 2000-2015; Barcelona 2011-2019; and, Istanbul 2016-2019 (see Figure 1). The choice of variables collected was at the discretion of the investigator(s), but included at least the following: patient demographic data; DFO diagnosis; DFO microbiology; DFO-related surgery; other relevant DFO therapies; and, DFO outcomes. The only consistently available antibiotic-resistance data were for the quinolone agents.

Statistical analyses

We used descriptive and comparative statistics (Pearson-χ^2 or Fisher's Exact test, Student-*t*, or Mann-Whitney-U-tests) and an un-

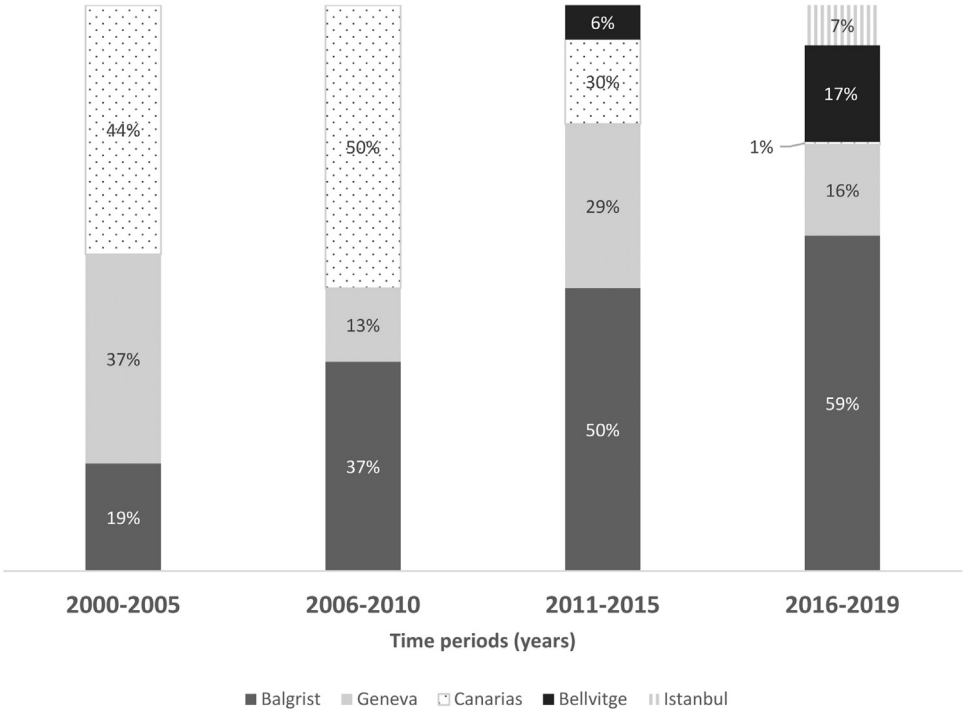

Figure 1. Percentage that each centre represents for each time period.

Table 1
Demographic characteristics of diabetic foot osteomyelitis episodes.

	Overall (n = 1379)	Zurich (n = 589)	Geneva (n = 323)	Las Palmas (n = 353)	Barcelona (n = 86)	Istanbul (n = 28)
Male sex (n;%)	1044 (76)	475 (81)	246 (76)	235 (67)	65 (76)	23 (82)
Median age (years, IQR)	67 (59-74)	68 (60-74)	69 (60-76)	65 (56-72)	63 (57-70)	67 (66-67)
Body mass index (median, kg/m^2)	29 (25-33)	30 (25-33)	27 (24-31)	not available	31 (26-40)	not available
Glycated hemoglobulin (%, median, IQR)	7.8 (6.8-9.2)	7.6 (6.6-8.7)	7.2 (6.4-8.8)	8.3 (7.1-9.9)	8.0 (7-9)	8.0 (7.1-9.8)
Type 2 diabetes mellitus (n;%)	1245 (90)	496 (84)	305 (94)	338 (96)	78 (91)	28 (100)
Years of diabetes mellitus (median, IQR)	17 (10-25)	18 (11-26)	14 (7-25)	18 (11-25)	10 (9-20)	20 (6-25)
Symptomatic arterial vasculopathy (n;%)	744 (54)	416 (71)	not available	209 (59)	37 (43)	not available
Diabetic neuropathy (n;%)	917 (67)	535 (91)	208 (64)	110 (31)	64 (74)	not available
≥1 previous foot infections (n;%)	634 (46)	260 (44)	167 (52)	142 (40)	37 (44)	not available
Forefoot osteomyelitis (n;%)	1136 (82)	497 (84)	235 (73)	321 (91)	62 (72)	21 (75)
Duration antibiotic therapy (median days, IQR)	21 (14-41)	21 (14-39)	25 (15-42)	not available	25 (0-40)	not available

Data presented are n (percentage), median values and interquartile ranges. The differences between cohorts were statistically significant, *P*-values were omitted.

conditional logistic regression analysis assessing for the large case-mix.

Because we had a large number of DFO episodes, and very few had missing data, we elected not to perform formal power analyses or imputations.

The multivariable logistic regression model controlled for key confounders, such as age, biological sex, and prior antibiotic treatment before microbiological sampling. It also assessed interactions between the variables 'Centre' and 'time period. We used STATA^{TM} software (16.1, College Station, USA) and classified *P*-values ≤ 0.05 (two-sided) as statistically significant.

Results

Study population

Among 3135 DFI from the composite database, we retrieved 1379 DFO cases: 589 (43%) from Zurich, 323 (23%) from Geneva, 353 (26%) from Las Palmas, 86 (6%) from Barcelona, and 28 (2%) from Istanbul (Table 1). Among all patients, 76% were male, the median age was 67 years (interquartile range [IQR], 59-74 years), 90% had type 2 diabetes, the median serum glycated haemoglobin level was 7.8% (IQR, 6.8%-9.2%) and the median diabetes duration was 17 years (IQR, 10-25 y). Patients experienced more than one DFO episode in 45% of cases, with 77% of these involving two episodes. Most DFO cases were anatomically localized to the forefoot (82%) and were treated with a combined surgical and medical approach in 73% of cases (1001 orthopaedic surgeries for the 1379 DFO episodes).

There were statistically significant differences between geographical cohorts in several characteristics. From a clinical point of view: i) the Barcelona cohort had the youngest patients and the shortest duration of diabetes mellitus (median 10 years, IQR 9-20); ii) patients from all cohorts had glycated hemoglobulin values at presentation of >7%, ranging from 7.2% in Geneva to 8.3% in Las Palmas; iii) cohorts from Zurich had a higher percentage of vasculopathy than those from Las Palmas and Barcelona; and, iv) a history of a previous foot infection ranged from 40% (in the Las Palmas) to 52% (in the Geneva) cohorts.

Microbiology

Among the 1379 culture-positive cases, polymicrobial infection occurred in 624 (45%). For simplicity, we interpreted every isolate from a bone sample as a true pathogen; thus, there were 2215 pathogens identified. Among them, 1454 cases (66%) were caused by gram-positive bacteria, 670 (30%) were gram-negative bacteria, and the remaining 75 by obligate anaerobes (3%), or fungi (n = 16, 1%).

S. aureus was the isolate most frequently cultured from the DFO cases (n = 644, 29%), among which 510 were methicillin-susceptible (MSSA) and 134 methicillin-resistant (MRSA) (representing 20.8% of all *S. aureus* isolates). Figure 2a shows the microbiology of gram-positive isolates.

Regarding gram-negative bacteria, the Enterobacteriaceae group was the most frequent (n = 444, 20%), whereas the most frequent responsible individual isolates were *Pseudomonas aeruginosa* (n = 161, 8%), *Enterobacter cloacae* (n = 103, 4.7%) and *Escherichia coli* (n = 100, 4.5%). Figure 2b shows the microbiology of Enterobacteriaceae group.

a) Differences in the microbiology among centres

Table 2 shows a comparison in the microbiology among the centres for the most common isolates. As the percentages in the Istanbul cohort may be biased by its small size, we did not refer to this cohort in further comparisons.

Overall, the proportion of gram-positive microorganisms was significantly higher in Zurich and Geneva than in Las Palmas and Barcelona. To the contrary, the proportion of gram-negative bacilli was significantly higher in both Spanish cohorts than in the Swiss cohorts. The proportion of *S. aureus* among all etiologic pathogens ranged from 35% in Zurich to 60% in Las Palmas ($P < 0.01$); among these isolates there was a higher proportion of MRSA in Barcelona (36%), Geneva (29%) and Las Palmas (24%) compared to Zurich (7%). The proportion of Enterobacteriaceae among all isolates was higher in Las Palmas and Barcelona (37% and 38%, respectively) than in Geneva and Zurich (28% and 21%, respectively; $P < 0.01$). The highest proportion of *P. aeruginosa* isolates was in the Barcelona cohort (30%).

b) Changing trends in microbiologic result among centres over time

To examine the trends, we grouped the total number of isolates from DFO episodes into four periods: 2000-2005 (P1), 2006-2010 (P2), 2011-2015 (P3) and 2016-2019 (P4).

Over the study period, the proportion of gram-positive microorganisms remained stable or decreased, particularly in Las Palmas from 83% in P1 to 65% in P3, $P = 0.03$; and, in Barcelona from 87% in P3 to 63% in P4, $P = 0.11$. The proportion of methicillin-susceptible *S. aureus* isolates did not change significantly, except in the Zurich cohort, where it increased from 21% in P1 to 35% in P4 ($P = 0.04$). The proportions of *Streptococcus* and *Enterococcus* remained stable as well, although these data are not shown in the table due to the minimal number of cases. However, there was a decrease in coagulase-negative staphylococci over the years in all cohorts. Additionally, the specific proportion of MRSA decreased over time in the Geneva (39% to 16%, $P = 0.03$) and Las Palmas (37% to 9%, $P \leq 0.01$), but remained stable in Barcelona. To the contrary, the proportion of gram-negative bacilli tended to increase in some cohorts, particularly in Enterobacteriaceae group, where the rise was more pronounced notably in Geneva from 16% in P1 to

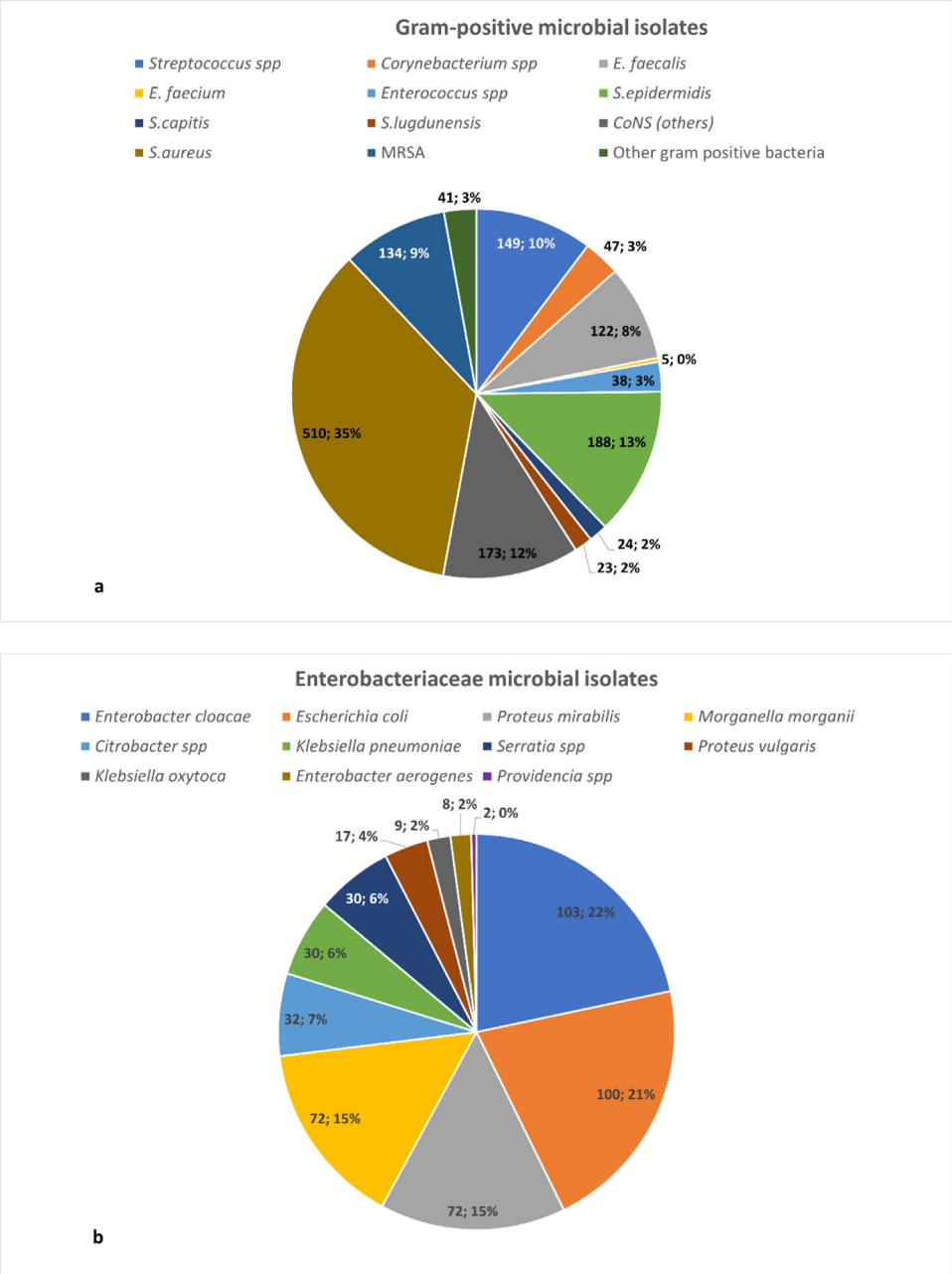

Figure 2. Microbiology of diabetic foot osteomyelitis, 2215 microbiologic isolates. **a**. Gram-positive isolates. **b**. Enterobacteriaceae group isolates.

39% in P4 ($P < 0.01$) and Las Palmas from 27% in P1 to 41% in P3 ($P < 0,01$) (See Table 3).

Quinolone resistance among gram-negative bacilli

We evaluated the available data on antibiotic resistance of a total of 369 bacterial isolates from Zurich, Geneva, and Barcelona. The overall rate of quinolone resistance was 12.5% (46/369 isolates), with 9% (9/103) in the first decade and 14% (37/266) in the second. Barcelona had the highest rate at 19%, compared to 14% in Geneva and 9% in Zurich. The isolates that were most frequently quinolone-resistant were *E. coli* and *P. aeruginosa* (25% for each) followed by *Enterobacter* spp. and *Proteus* spp. (13% each).

Specifically, for *Pseudomonas aeruginosa* and Enterobacteriaceae, the MIC threshold for quinolone resistance was set at >0.5 mg/L for ciprofloxacin, as per EUCAST criteria (https://www.eucast.org/clinical_breakpoints).

Risk associations for enterobacterial osteomyelitis

Table 4 shows the results of the univariate and multivariate logistic regression analyses. The proportion of Enterobacteriaceae was not significantly associated with the patient's age or prior antibiotic treatment. In contrast, the presence of necrosis (or ischemic maceration) was significantly associated with a higher proportion of Enterobacteriaceae compared to other bacterial groups (odds ratio [OR] 1.65, 95% confidence interval [CI] 1.26-2.15; $P < 0.01$).

Using the first study period (2000-2005) and the cohort from Zurich as the references in the multivariate analysis, we found that all other cohorts were at a higher risk for Enterobacterial DFO, in-

Table 2
Microbiological results by centre for each episode.

	Zurich (n = 589)	Geneva (n = 323)	Las Palmas (n = 353)	Barcelona (n = 86)	Istanbul (n = 28)
Gram positive bacteria (n = 1130)	514 (87)	283 (88)	262 (74)	58 (67)	13 (46)
Staphylococcus aureus (n = 644)	204 (35)	188 (58)	212 (60)	36 (42)	4 (14)
MSSA (n = 510)	189 (32)	133 (41)	162 (46)	23 (27)	3 (11)
MRSA[a] (n = 134)	15 (7)	55 (29)	50 (24)	13 (36)	1 (25)
Streptococcus spp (n = 137)	56 (10)	59 (18)	13 (4)	7 (8)	2 (7)
Enterococcus spp (n = 162)	90 (15)	48 (15)	5 (1)	14 (16)	5 (18)
CoNS (n = 368)	255 (43)	63 (20)	38 (11)	8 (9)	4 (14)
Staphylococcus epidermidis (n = 183)	129 (22)	13 (4)	38 (11)	5 (6)	3 (11)
Gram negative i(n = 568)	178 (30)	134 (41)	179 (51)	56 (65)	21 (75)
Enterobacteriaceae (n = 394)	123 (21)	90 (28)	132 (37)	33 (38)	16 (57)
Escherichia coli (n = 100)	27 (5)	19 (6)	39 (11)	9 (10)	6 (21)
Enterobacter cloacae (n = 103)	38 (6)	28 (9)	29 (8)	6 (7)	2 (7)
Pseudomonas aeruginosa (n = 161)	49 (8)	49 (15)	30 (9)	26 (30)	7 (25)
Obligate anaerobes (n = 74)	46 (8)	27 (8)	1	0	0

Data are presented for episodes with specific microbiological findings. (n; percentage).
[a] The percentages of MRSA are out of the total of *Staphylococcus aureus* isolates.
MSSA, Meticillin sensitive *Staphylococcus aureus*; MRSA, Meticillin resistant *Staphylococcus aureus*; CoNS : Coagulase negative staphylococci.

Table 3
Rates of centre-specific microbiologic results over a specific time periods.

Episode / centre (n/N, %)		Time Period				
		2000-2005 (n = 303)	2006-1010 (n = 333)	2011-2015 (n = 336)	2016-2019 (n = 377)	*P* value
GPB	Zurich (n = 589)	50/57 (88)	101/122 (83)	153/168 (91)	210/242 (87)	0.22
	Geneva (n = 323)	101/113 (89)	37/44 (84)	87/99 (88)	58/67 (87)	0.83
	Las Palmas (n = 351)	110/133 (83)	118/167 (71)	33/51 (65)	-	0.03
	BCN (n = 86)	-	-	15/18 (87)	43/68 (63)	0.11
MSSA	Zurich (n = 589)	12/57 (21)	30/122 (25)	63/168 (38)	84/242 (35)	0.03
	Geneva (n = 323)	40/113 (35)	19/44 (43)	42/99 (42)	32/67 (48)	0.41
	Las Palmas (n = 351)	53/133 (40)	79/167 (47)	29/51 (57)	-	0.21
	BCN (n = 86)			6/18 (33)	17/68 (25)	0.48
MRSA[a]	Zurich (n = 204)	0/12	6/36(17)	2/65 (3)	7/91 (8)	0.09
	Geneva (n = 181)	24/61 (39)	12/28 (43)	13 /55 (24)	6/37 (16)	0.03
	Las Palmas (n = 211)	31 /84 (37)	16 /94 (17)	3/32 (9)	-	<0.01
	BCN (n = 36)	-	-	2/8 (25)	11/28 (39)	0.68
CoNS	Zurich (n = 589)	33/57 (58)	55/122 (45)	77/168 (46)	90/242 (37)	0.03
	Geneva (n = 323)	30/113 (27)	4/44 (9)	17/99 (17)	12/67 (18)	0.07
	Las Palmas (n = 351)	22/133 (17)	16/167 (10)	-	-	0.01
	BCN (n = 86)	-	-	4/18 (22)	4/68 (6)	0.03
GNB	Zurich (n = 589)	14/57 (25)	35/122 (29)	54/168 (32)	75/242 (31)	0.71
	Geneva (n = 323)	35/113 (31)	19/44 (43)	47/99 (47)	33/67 (49)	0.04
	Las Palmas (n = 351)	58/133 (43)	93/167 (56)	27/51 (53)	-	0.21
	BCN (n = 86)	-	-	9/18 (50)	47/68 (69)	0.13
Enterobacteriaceae	Zurich (n = 589)	12/57 (21)	15/122 (12)	38/168 (23)	58/242 (24)	0.07
	Geneva (n = 323)	18/113 (16)	13/44 (30)	33/99 (33)	26/67 (39)	<0.01
	Las Palmas (n = 351)	36/133 (27)	75/167 (45)	21/51 (41)	-	<0.01
	BCN (n = 86)	-	-	4 /18 (22)	29/68 (43)	0.11

Overall, 1349 episodes were analysed in the comparisons. However, 2 episodes from Las Palmas in the last period were deemed non-representative and excluded from the table, along with 28 episodes from Istanbul in the same period, as they did not allow for meaningful comparisons.
n represents the number of episodes for each centre within the specified time period, while N represents the total number of episodes for each centre in the same period.
[a] MRSA is reported as n/N (%), where N refers to the total number of *Staphylococcus aureus* episodes.
GPB: Gram-positive bacteria; MRSA: Methicillin-resistant *Staphylococcus aureus*; CoNS: Coagulase-negative staphylococci; GNB: Gram-negative bacilli.

cluding Geneva. However, the Spanish cohorts, and the last time period, were each particularly associated with Enterobacterial bone infection; the strongest association was with Las Palmas (OR 3.14, 95% CI 2.19-4.50, $P < 0.01$), and the last time period (OR 2.68, 95% CI 1.75-4.10).

Discussion

Effective treatment of DFO depends on identifying the causative pathogens and their antibiotic sensitivities. Historically, gram-positive cocci, especially *S. aureus* [2,4], have been the main pathogens. However, aerobic gram-negative bacilli are also common, particularly in ischemic vasculopathy and chronic wounds, and are more prevalent in Asia and North Africa. Our study, covering cohorts from five European centres, offers valuable insights into the causative microorganisms in community-acquired DFO over time.

Over the last two decades, there has been a rise in infections caused by Enterobacteriaceae, while the prevalence of infections caused by gram-positive has remained stable. Epidemiological differences were also observed among our cohorts, with a higher incidence of MRSA infections in Spain and a lower, stabilized incidence in Zurich [21]. Interestingly, MRSA cases have decreased significantly since 2005 in hospitals in both the United States and Europe. In our study, MRSA accounted for 28% of all *S. aureus* infections from 2000 to 2010, but this decreased to 14% from 2011 to 2020.

We believe our findings accurately reflect the current microbiology of DFO, being largely free from major selection bias and supported by previous studies [10,11,17,22]. The increase in infec-

Table 4
Multivariate logistic regression regarding the microbiological detection of *Enterobacteriaceae* osteomyelitis.

	Enterobacterial OM (n = 378, 28%)	Non-Enterobacterial OM (n = 973, 72%)	Univariate results OR (95% CI)	Multivariate results OR (95% CI)	*P*-value
Age (years)	66 (58-74)	67 (59-74)	0.99 (0.99-1.01)	0.99 (0.99-1.01)	0.74
Male sex	284 (75)	737 (76)	0.97 (0.73-1.27)	1.03 (0.77-1.37)	0.84
Antibiotic therapy prior to admission	203 (54)	577 (59)	0.80 (0.63-1.01)	0.88 (0.67-1.15)	0.35
Presence of ischemic necrosis	127 (34)	235 (24)	1.59 (1.23-2.06)	1.65 (1.26-2.15)	<0.01
Study centres					
Zurich	123 (33)	466 (48)	1 (default)	1 (default)	
Geneva	90 (24)	233 (24)	1.46 (1.07-2.00)	1.77 (1.24-2.53)	<0.01
Barcelona	33 (9)	53 (5)	2.36 (1.46-3.80)	2.03 (1.22-3.39)	<0.01
Las Palmas-Canarias	132 (35)	221 (23)	2.26 (1.69-3.03)	3.14 (2.19-4.50)	<0.01
Study periods in years					
2000-2005	66 (17)	237 (24)	1 (default)	1 (default)	
2006-2010	103 (27)	230 (24)	1.61 (1.12-2.30)	1.77 (1.21-2.58)	<0.01
2011-2015	96 (25)	240 (25)	1.44 (1.00-2.06)	2.06 (1.39-3.04)	<0.01
2016-2019	113 (30)	266 (27)	1.53 (1.07-2.17)	2.68 (1.75-4.10)	<0.01

Results expressed as odds ratios (OR), 95% confidence intervals and rounded *P*-values.

tions caused by gram-negative and antibiotic-resistant pathogens, particularly noted in the Mediterranean basin, presents significant challenges for managing DFO [8,11,14,23].

Initially observed in nosocomial settings, the increasing prevalence of gram-negative infections has now spread to community settings. European Antimicrobial Resistance Surveillance Reports indicate high resistance rates in clinical isolates of *E. coli* and K. *pneumoniae*, with notable quinolone resistance. For example, fluoroquinolone resistance of invasive *E. coli* rose from 20% in 2009 to 23.8% in 2019 [24,25]. Switzerland saw a rise in quinolone-resistant *E. coli* from 10.3% in 2004 to 19.4% in 2015 [26]. A 10-year retrospective cohort study from Turkey reported an increased incidence of gram-negative bacilli and antimicrobial resistance in DFI, particularly ESBL-producing *E. coli* [12].

Globally, there has also been a rising trend in Gram-negative resistance. In a recent study from Peru, 60% of DFO isolates were quinolone-resistant [11]. In Egypt, carbapenem resistance was found in 14% of DFI isolates [21], and in Lebanon ESBL strains accounted for 25% of DFO cases, with 37% showing quinolone resistance [23].

Our study revealed an increase in quinolone-resistant gram-negative bacilli in DFO cases, from 9% in the first decade to 14% in the second. Significant differences were noted between centres, with Barcelona having the highest rate (19%) and Geneva showing almost double the rate compared to Zurich (14% vs. 9%). *E. coli* and K. *pneumoniae* were the most frequently identified species among quinolone-resistant gram-negative bacilli.

These findings emphasize the need for antibiotic stewardship [20]. Overuse of antibiotics in both human and veterinary medicine is a major factor contributing to increasing antibiotic resistance [27]. Key risk factors for gram-negative DFO include the presence of necrosis in the wound and infections occurring in more recent time periods. A Chinese retrospective study [28] linked prolonged antibiotic exposure and diabetes duration with higher risks of gram-negative foot infections, though it remains unclear if antibiotic-resistant pathogens were more prevalent in subsequent DFI episodes [28–30]. Non-antibiotic factors also play a role, such as aging populations with advanced peripheral arterial disease [29] leading to chronic lower limb ischemia and maceration in pressure areas, which is common in calcaneal DFO and often associated with infection with *Pseudomonas* [8]. Other studies have confirmed that ischemic and pressure-related necrosis are risk factors for gram-negative infections in diabetic feet [10,18,29].

Geographic location impacts the risk of gram-negative DFO. In warm, humid climates, environmental microorganisms can invade through ulcers, particularly in areas with ritual foot washing, poor hand hygiene in medical settings, and use of non-protective footwear. These factors likely contribute to the higher rates of gram-negative DFO observed in Mediterranean regions compared to Central Europe in our study [1].

Our study's strength lies in the comprehensive inclusion of patients from five academic centres with established DFO cohorts and scientific expertise. Limitations of the study include: some risk for inherent selection bias; focus on only specific pathogen groups; inclusion of predominantly bone infections; and, exclusive inclusion of selected countries. Conducting prospective surveillance across Europe over several decades would provide more universally applicable data. We hope our findings will encourage further investigation into risk factors for gram-negative and antibiotic-resistant infections in patients with DFO.

We are witnessing a rapid increase in gram-negative and quinolone-resistant pathogens in community-acquired DFO cases, especially in Spain, Turkey, and to some extent in Switzerland. We urge healthcare providers to follow antibiotic stewardship principles to counter this trend [20].

Author contributions

Conceptualization: L.S.B., I.U., F.W.A.W., B.A.L., B.E., J.A.S., K.G., and O.M.; Methodology: L.S.B., F.W.A.W., I.U., and M.S; Validation: L.S., I.U., F.W.A.W., M.S., A.F., and M.S; Investigation: L.S., I.U; Resources: M.S., F.W.A.W., I.U., and O.M; Data curation: L.S.B., I.U., B.E., K.G., D. L., and J.A.S; Data collection: A.N., M.C.B., A.F., M.S., K.G., D. L., and I.U.; Data analysis: L.S.B and I.U.; Analysis verification: I.U. Writing: L.S.B., K.G., F.W.A.W., M.S., B.E:, J.A.S:, O.M., B.A.L.;.Writing review and editing, L.S:B., B.A.L., and I.U; Visual Supervision: I.U., F.W.A.W., and M.S; Project administration: L.S., and I.U. All authors read and approved the final manuscript.

Informed consent statement

The responsibility for obtaining patient consent was at the discretion of each participating centre. As a principle, all included patients had a generic informed consent form signed, or they participated on other studies in the field of DFO, for which they authorized us to use their data in an anonymous form and in accordance with the regulations. This cohort project was approved by the local Ethics' Committee of Zurich Canton, Switzerland (BASEC–Number 2019-01994).

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Congress participation

Preliminary parts of this manuscript have been presented at the 7th National Diabetic Foot Infection Symposium 12-15 October 2022 in Kemer, Antalya, Turkey (1st prize for epidemiological research) and at the 9th International Symposium on the Diabetic Foot 10-13 May 2023 | The Hague - The Netherlands.

Declaration of competing interest

The authors have no competing interests to declare.

Acknowledgements

We are indebted to the study nurses of the Unit for Clinical and Applied Research (UCAR) at Balgrist University Hospital for their help.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2025.107843.

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