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Risk factors for methicillin-resistant *Staphylococcus aureus* and extended-spectrum ß-lactamase-producing Enterobacterales in patients with diabetic foot infections requiring hospital admission

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ABSTRACT

Purpose. Methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum β-lactamase-producing Enterobacterales (ESBL-E) may complicate the treatment of diabetic foot infections (DFIs). The aim of this study was to determine the risk factors for these pathogens in DFIs.

Material and methods. This was a prospective observational study of 167 consecutive adult patients with DFIs. The diagnosis and severity of DFIs were based on the Infectious Disease Society of America (IDSA) classification system. Multivariate analyses were performed in order to identify risk factors for MRSA and ESBL-E infections.

Results. *S. aureus* was the most isolated pathogen (n= 82, 37.9 %) followed by *Escherichia coli* (n= 40, 18.5%). MRSA accounted for 57.3% of all *S. aureus* and 70% of *Klebsiella pneumoniae* and 25% of *E. coli* were ESBL producers, respectively. Deep ulcer [OR 8,563; 95% CI (1,068-4,727)], previous use of fluoroquinolones [OR 2,78; 95% CI (1,156-6,685)] and peripheral vasculopathy [OR 2,47; 95% CI (1.068-4.727)] were the independent predictors for MRSA infections; and osteomyelitis [OR 6,351; 95% CI (1,609-25,068)] and previous use of cephalosporins [OR 5,824; 95% CI (1,517-22,361)] for ESBL-E infections.

Conclusions. MRSA and ESBL-E have adquired a great clinical relevance in DFIs. The availability of their risk factors is very convenient to choose the empirical treatment in severe forms.

Key words: diabetic foot infection, methicillin-resistant *Staphylococcus aureus*, ESBL-producing Enterobacterales, risk factors, hospital admission

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Factores de riesgo de infección por *Staphylococcus aureus* resistente a meticilina y enterobacterias productoras de betalactamasas en infecciones de pie diabético que requieren hospitalización

RESUMEN

Objetivo. *Staphylococcus aureus* resistente a meticilina (MRSA) y las enterobacterias productoras de betalactamasas (ESBL-E) pueden complicar el tratamiento de las infecciones del pie del diabético (DFIs). El objetivo de este estudio fue determinar los factores de riesgo de las infecciones por estos microorganismos en el pie del diabético

Material y métodos. Estudio observacional prospectivo de 167 pacientes consecutivos con infecciones del pie del diabético. El diagnóstico y gravedad de las infecciones se basó en la guía de la *Infectious Disease Society of America* (IDSA). Para identificar los factores de riesgo de las infecciones por MRSA y (ESBL-E) se llevó a cabo mediante un estudio multivariante.

Resultados. *S. aureus* fue el microorganismo más aislado (n= 82; 37,9 %) seguido por *Escherichia coli* (n= 40; 18,5%). El 57,3% de *S. aureus* fueron MRSA y el 70% de *Klebsiella pneumoniae* y el 25% de *E. coli* eran productores ESBL, respetivamente. Los factores de riesgo independientes de las infecciones por MRSA fueron las úlceras profundas [OR 8,563; IC 95% (1,068-4,727)], uso previo de fluoroquinolonas [OR 2,78; IC 95% (1,156-6,685)] y la vasculopatía periférica [OR 2,47; IC 95% (1.068-4.727)], mientras que para las infecciones por (ESBL-E) lo

fueron osteomielitis [OR 6,351; 95% IC 95% (1,609-25,068)] y el uso previo de cefalosporinas [OR 5,824; IC 95% (1,517-22,361)].

Conclusiones. MRSA y ESBL-E han adquirido una gran relevancia clínica en las DFIs. La disponibilidad de sus factores de riesgo es muy conveniente para elegir el tratamiento empírico en las formas graves.

Palabras clave: Infección del pie diabético, *Staphylococcus aureus* resistente a meticilina, enterobacterias productoras de ESBL, factores de riesgo, hospitalización

INTRODUCTION

Diabetic foot infections (DFIs) along with ischemia are the main underlying factors contributing to lower-extremity amputation in the United States and Europe [1,2]. The relative frequencies of microorganisms causing wound infections varying greatly among studies, type and severity of lesions, and geographic area [3]. Monomicrobial infections by aerobic gram-positive cocci (*Staphylococcus aureus* and *Streptococcus* spp.) are predominant organisms in acute and untreated ulcers, by contrast, chronic wounds infections are more frequently polymicrobial (aerobic Gram-positive cocci, Gram-negative bacilli and anaerobes) [3]. *S. aureus* is the most frequently isolated microorganism in diabetic foot ulcers in Spain, followed by Enterobacterales [1,4]. In addition, more than 30% of *S. aureus* are methicillin-resistant (MRSA) [4] and colonization or infection of chronic ulcers by MRSA can result in bacteremia between 8% and 22%, that is associated with a 30-day mortality of about 30% [5]. DFIs by extended-spectrum β-lactamase-producing Enterobacterales (ESBL-E) have been also described, but are less frequent in our environment [4,6,7]. Despite this, the information about multidrug-resistant organisms (MDROs) such as MRSA and ESBL-E as a cause of DFIs in patients requiring hospital admission, is not yet enough [8,9]. The emergence of MDROs can complicate the treatment of DFIs, and may even cause a worse course of the injury [10]. The aim of this study was to determine the bacterial profile and risk factors for MRSA and ESBL-E in patients with DFIs requiring hospital admission.

MATERIAL AND METHODS

A single-institutional prospective observational study was performed with the inclusion of all consecutive adult diabetic patients with infected foot ulcers admitted to the Infectious Disease Department or General Surgery Department of Hospital Clinico Universitario Virgen de la Arrixaca in Murcia (Spain) from 2013 to 2017 for acute DFIs. The study was approved by the ethics committee of the hospital before conducting it (reference 2013-10-10-HCUVA). The patients were included after obtaining informed consents. The diagnosis and severity of DFIs were based on the Infectious Disease Society of America (IDSA) classification system. Diabetic foot ulcers were also classified into three groups: 1) neuropathic lesions, 2) ischemic lesions and 3) mixed or neuro-ischemic lesions. Diabetic foot ulcers with infection involving skin and subcutaneous tissues were considered as deep ulcer [11]. Demographic data, hospitalization and antibiotic therapy within the previous 3 months, nursing home residence and underlying illnesses were recorded. A clinical evaluation including ulcer size and depth and neurological and vascular status was performed. Microbiological, laboratory, and radiographic evaluations were carried out during hospitalization, in keeping with the routine hospital practice. After washing surface of the ulcer with saline solution, three to five cultures were obtained at the time of admission by curetted material at the bottom of the wound, and bone biopsy was performed when osteomyelitis was suspected. Bacteria were isolated and identified by standard methods. Antimicrobial susceptibility testing of the isolates was performed by an automated system VITEK® 2 (bioMérieux, marcy l´etoile, France) with AFTN 112 cards. ESBL-producing strains were phenotypically identified according to Clinical and Laboratory Standards Institute (CLSI) recommendations [12]. Obesity was defined according to body mass index criteria [13]. Glomerular filtration rate was estimated from serum creatinine using the equation of Cockcroft-Gault [14]. Toronto Consensus Panel on Diabetic Neuropathy was used for diagnosis of diabetic neuropathy [15]. Diabetic retinopathy was divided in two major forms: nonproliferative (mild and moderate-severe) and proliferative by the absence or presence of abnormal new blood vessels in the retina, respectively [16]. Patients were treated according to the hospital protocol with parenteral antibiotics together with concomitant surgical debridement, revascularization (bypass), and/or reconstruction (skin graft) techniques.

Statistical analysis was performed using SPSS version 18.0 software (SPSS Inc, Chicago, IL). Quantitative variables were expressed by mean ± standard deviation, and qualitative variables by percentages. Significance was determined by the $\chi 2$ test and Fisher´s exact test, when necessary, for qualitative variables, and by t test or U-Mann-Whitney non-parametric tests, when necessary, for quantitative variables. Significance level was established at $p \leq 0.05$. A stepwise logistic regression multivariate analyses was performed in order to identify risk factors for MRSA and ESBL-E infections. All variables showing differences in bivariate analyses ($p < 0.1$) were considered for inclusion in the multivariate model.

RESULTS

The study included 167 consecutive diabetic patients with foot infections. Swab samples from the bottom of the ulcer were taken in all cases and bone biopsy was performed in 82 (49%). A total of 216 microorganisms were isolated. *S. aureus* was the most isolated pathogen (n= 82, 37.9%) followed by *Escherichia coli* (n= 40, 18.5%). Other *Enterobactericeae* other than *E. coli* (n= 45, 20.8%) and *Pseudomonas aeruginosa* (n= 12, 5.4%) were also common (Table 1). The number of aerobic gram-positive cocci was over aerobic gram-negative bacilli globally (110/100) and in samples taken from bone (51/35), but not in ulcers (60/65). Infections were polymicrobial in 95 cases (56.8%). Regarding bacterial resistance, 57.3% of *S. aureus* were MRSA (n=27, 57.4% in ulcer) and 25% of *Enterobacte-*

Table 1 **Isolated microorganisms from ulcer and bone**

Microorganisms	Total no. (%)	Ulcer no. (%)	Bone no. (%)
Staphylococus aureus	82 (37.9)[a]	46 (56.9)	36 (43.9)
Escherichia coli	40 (18.5)[b]	26 (65)	14 (35)
Streptococcus pyogenes	14 (6.4)	8 (57.1)	6 (42.8)
Enteroccus faecalis	13 (6)	5 (38.5)	8 (61.5)
Pseudomonas aeruginosa	12 (5.5)	9 (75)	3 (25)
Morganella morganii	12 (5.5)	5 (41.7)	7 (58.3)
Proteus mirabilis	11 (5)	10 (90.9)	1 (9.1)
Klebsiella pneumoniae	10 (4.6)[c]	3 (30)	7 (70)
Enterobacter cloacae	7 (3.2)	6 (85.7)	1 (14.3)
Klebsiella oxytoca	3 (1.3)	3 (100)	0
Acinetobacter baumanii	3 (1.3)	1 (33.3)	2 (66.7)
Providencia stuartii	2 (0.9)	2 (100)	0
Bacteroides urealyticus	3 (1.3)	3 (100)	0
Staphylococcus hominis	1 (0.4)	1 (100)	0
Peptostreptococcus spp	1 (0.4)	1 (100)	0
Clostridium perfringens	1 (0.4)	0	1 (100)
Candida albicans	1 (0.4)	1 (100)	0
Total	216 (100)	130 (60.1)	86 (39.8)

[a]MRSA: 47/82 (57.3%), 27 (57.4%) ulcer; [b,c]ESBL-producing Enterobacterales: 17/68 (25%), 16/17 (94%) in bone, *E. coli* 10/40 (25%), *K. pneumoniae* 7/10 (70%)

riceae were ESBL producers (70% of *Klebsiella pneumoniae*, 25% of *E. coli* and 94% in bone) (Table 1). In addition, 25% of *E. coli* were resistant to ciprofloxacin, 22.5% to amoxicillin/clavulanic acid and 7.5% to carbapenems. *P. aeruginosa* shows resistance to ciprofloxacin (75%), piperacillin/tazobactam (75%), and carbapenems (50%). Surgical drainage and/or debridement was performed in all patients. One hundred (60%) patients need digital amputation and 13 (7.8%) major amputation. The most frequently used antibiotic regiments were: clindamycin plus piperacillin-tazobactam, cefepime or ertapenem (n= 118; 70.6%) and linezolid plus meropenem (n= 47; 28.1%).

Table 2 shows patient's characteristics, comorbidities, location of infection and severity distributed by main causative agents and development of antimicrobial resistance or not, respectively. The mean age of the subjects was 62.9+12.1 years and 77.6% of the patients were male. The mean time of diabetes evolution was 20+8.39 years and the mean of length of stay (LOS) 17.08+10.11 days. All patients had a Charlson index >3, 90% neuropathy, 85% deep ulcer and 91% previous ulcer. Moderate infections were present in 118 (70.7%) patients and osteomyelitis in 69 (41.3 %).

In the univariate analysis, smoking (p= 0.002), obesity (p= 0.05), proliferative retinopathy (p= 0.023), peripheral vasculopathy (p= 0.05), wound size > 2 cm^2 (p= 0.002), deep wound (p= 0.022), left wounds (p= 0.04) and previous use of fluoroquinolones (3 months before) (p= 0.019) were the variables significantly associated with MRSA infections, while use of cephalosporins (p= 0.005), wound size > 2 cm^2 (p= 0.04), severity of infection (p= 0.05) and osteomyelitis (p= 0.012) were associated with ESBL-E infections (Table 2).

In the multivariate analysis, deep ulcer [OR 8,563; 95% CI (1,068-4,727)], previous use of fluoroquinolones [OR 2,78; 95% CI (1,156-6,685)] and peripheral vasculopathy [OR 2,47; 95% CI (1.068-4.727)] were the independent predictors for MRSA infections; and osteomyelitis [OR 6,351; 95% CI (1,609-25,068)] and previous use of cephalosporins (3 months before) [OR 5,824; 95% CI (1,517-22,361)] for ESBL-E infections (Table 3).

DISCUSSION

As in previous studies performed in Spain and in other industrialized countries, *S. aureus* continues to be the most common isolated pathogen in DFIs, followed by *E. coli* and other *Enterobactericeae*. Overall, about 75% of DFIs in Spain are due to *S. aureus* and *Enterobactericeae* [1,4] and the empiric treatment should consider their current rates of resistance. MRSA remains above 30%, but ESBL-E (25% globally), particularly *K. pneumoniae*, have emerged as a serious and common problem in patients with diabetic foot ulcer that is

Table 2 **Patient's characteristics, comorbidities, location of infection and severity distributed by main causative agents and development of antimicrobial resistance or not, respectively. Data expressed as no. (%) or mean ± SD**

	Overall n= 167	MSSA n= 35	MRSA n= 47	p	E n= 68	ESBL-E n= 17	p
Male sex, no. (%)	133 (79.6)	28 (80)	35 (74.4)	0.376	57 (83.8)	13 (76.4)	0.346
Age (years), mean+SD	62.6+12.1	62.4+15.4	60.1+14.9	0.645	53.3+15.6	65.7+12.3	0.558
LOS (days), mean+SD	17.08+10.1	15.8±8.7	18.36±11.2	0.872	7.4±11.3	32.6±8.8	0.234
Diabetes evolution (years), mean+SD	20.08+8.39	20.6±3.5	22.7±7.1	0.22	19.7±8.06	19.7±9.01	0.379
Diabetes type 2, no. (%)	149 (89.2)	32 (91.4)	43 (91.4)	0.645	60 (88.2)	14 (82.3)	0.382
Diabetes treatment, no. (%)							
OAA	19 (11.3)	4 (11.4)	1 (2.1)	0.14	7 (10.2)	7 (41.1)	0.87
Insulin+OAA	148 (88.6)	31 (88.5)	44 (93.6)	0.21	61 (89.7)	10 (58.8)	0.41
Glycated hemoglobin[a], no.(%)	112 (67)	27 (77.1)	31 (65.9)	0.124	51 (75)	13 (76.4)	0.403
Smoking, no. (%)	83 (49.7)	16 (45,7)	38 (80.8)	0.002	22 (32.3)	7 (41.1)	0.576
Obesitiy, no. (%)	51 (30.5)	16 (45,7)	32 (68)	0.05	33 (48.5)	7 (41.1)	0.640
Hypertension, no. (%)	136 (81.4)	16 (45,7)	44 (93.6)	0.119	53 (77.9)	10 (58.8)	0.09
Vasculopathy, no. (%)	51 (30.5)	18 (51.4)	38 (80.8)	0.05	37 (54.4)	10 (58.8)	0.161
Neuropathy, no. (%)	151 (90.4)	31 (88.5)	45 (95.7)	0.157	59 (86.7)	16 (94.1)	0.614
Retinopathy, no. (%)							
Mild	8 (4.7)	1 (2.8)	1 (2.1)	0.365	3 (4.4)	3 (17.6)	0.103
Moderate-severe	70 (41.9)	14 (40)	16 (34)	0.246	33 (48.5)	7 (41.1)	0.636
Proliferative	56 (33.5)	21 (60)	36 (76.5)	0.023	-	-	-
Renal insufficiency, no. (%)							
Grade 1	14 (8.3)	2 (5,7)	3 (6,3)	0.349	3 (4.4)	6 (35.2)	0.358
Grade 2	53 (31.7)	14 (40)	16 (34)	0.332	29 (42.6)	1 (5.8)	0.186
Grade 3	39 (23.3)	3 (8.5)	8 (17)	0.392	21 (30.8)	7 (41.1)	0.259
Grade 4	23 (13.7)	5 (14.2)	7 (14.8)	0.173	8 (11.7)	3 (17.6)	0.331
Prior infection[b], no. (%)	152 (91)	31 (88,5)	35 (74.4)	0.210	61 (89.7)	15 (88.2)	0.575
Prior antibiotics[b], no. (%)	113 (67.6)	21 (60)	39 (82.9)	0.019	48 (70.5)	15 (88.2)	0.005
Ulcer, no. (%)							
Forefoot	122 (73)	29 (82.8)	29 (61.7)	0.111	52 (76.4)	12 (70.5)	0.413
Size > 2 cm^2	96 (57.4)	17 (48.5)	38 (80.8)	0.002	38 (55.8)	13 (76.4)	0.04
Deep[c]	143 (85.6)	29 (82,8)	46 (97.8)	0.022	57 (83.8)	11 (64.7)	0.081
Mixed	108 (64.6)	25 (71.4)	28 (59.5)	0.191	45 (66.1)	10 (58.8)	0.383
Supuration	112 (67)	25 (71.4)	29 (61.7)	0.248	45 (66.1)	13 (76.4)	0.306
Fetid odor	108 (64.6)	24 (68.5)	28 (59.5)	0.274	46 (67.6)	10 (58.8)	0.339
Left foot	78 (46.7)	15 (42.8)	32 (68)	0.04	24 (35.2)	7 (41.1)	0.444
Osteomyelitis, no. (%)	69 (41.3)	16 (45.7)	20 (42.5)	0.563	30 (44.1)	16 (94.1)	0.012
Severity infection, no. (%)							
Moderate	108 (64.6)	28 (80)	33 (70.2)	0.66	41 (60.2)	6 (35.2)	0.483
Severe	57 (34.1)	7 (20)	12 (25.5)	0.558	27 (39.7)	11 (64.7)	0.05
McCabe, no. (%)							
Nonfatal	142 (85)	31 (88.5)	33 (73.3)	0.754	64 (94.1)	14 (82.4)	0.401
Ultimaly fatal	23 (13.7)	4 (11,4)	12 (25.5)	0.435	3 (4.4)	3 (17.6)	0.103

MSSA: methicillin-susceptible *S. aureus*; MRSA: methicillin-resistant *S. aurerus*; E: non ESBL-producing Enterobacterales; ESBL-E: ESBL-producing Enterobacterales; LOS: Length of stay; OAA: oral antidiabetic agents; [a]> 7%; [b]Exposure within 3 previous months; [c]erythema > 2 cm, or involving structures deeper than skin and subcutaneous tissues

consistent with the prevalence of these organisms in our environment [3,4,17]. Infections caused by MDROs are associated with higher morbidity and mortality than those caused by their susceptible counterparts [10], however, their coverage is not always suitable in all cases from ecologically and economically. Risk factors for MDROs infection are often common and include prior colonization, infection and use of antimicrobials, recent hospitalization, nursing-home residence and underlying diseases (diabetes mellitus, chronic renal failure in program of dialysis and hypoproteinemia) [18,19].

Previous retrospective and prospective studies in diabetic foot ulcers have also identified some risk factors for MRSA colonization or infection and for ESBL-E infection, such as wound size, osteomyelitis, history of MRSA foot colonization or infection, nasal carriage of MRSA, colonization and infection by others MDROs, prior use of antibiotics, long course of ulcer, chronic kidney disease, proliferative retinopathy, hypertension and poor glycemic control [6,8,20-26]. In the present prospective study with 167 patients included, we have identified three risk factors for MRSA infection (deep ulcer, prior treatment with fluoroquinolones and peripheral vasculopathy) that could explain 71.8% of them, and two for ESBL-E infection (osteomyelitis and previous use of cephalosporins), confirming some findings already described. The depth (tissue loss) of ulcer, one of the criteria used to develop the PEDIS system [27] and a recommendation from the IDSA to assess the DFIs [11], is the first time described as a risk factor for MRSA infection. However, there was no association between severity of lesion and MRSA infection. Peripheral vasculopathy neither has been identified as another risk factor for MRSA infection, but as a more common underlying condition [22]. Previous use of antibiotics was another predictive risk factor observed (fluoroquinolones for MRSA infections and cephalosporins for ESBL-E infections). The association between antibiobic exposure and MDROs has been frequently reported in DFIs and elsewhere [8,18,23,24,28,29]. The use of antimicrobial agents in diabetic foot ulcers, often excessive and unnecessary, can facilitate conditions in which bacteria with mechanisms of resistance experience a competitive advantage [18,25]. The mechanism for fluoroquinolones and cephalosporins to select MDROs remains unclear, but some authors believe it could be by selective inhibition or by killing of the more susceptible bacterial populations [18]. So far, osteomyelitis had only been recognized as a risk factors for MRSA infection in diabetic patients [8,22-24]. This finding in ESBL-E can help to know their risk factors, of which there are few data, mostly from Indian studies [6-8]. This fact is not surprising since gran-negative bacilli is an important cause of diabetic foot osteomyelitis and they have been associated with wounds caused by traumatic injury [30]. From a practical point of view, interventions directed at preventing the transmission of MDROs between diabetic patients and to reduce the inappropriate use of fluoroquinolones and cephalosporins in DFIs, should be attempted, as only modifiable variables.

Although this is one of the largest prospective series of DFIs to know risk factors for MDROs, the study was performed in a single centre, whose local epidemiology may limit the conclusions. However, the microbiological profile found in our series is very similar to that other previous Spanish studies [1-4].

In conclusion, MRSA and ESBL-E have currently adquired a great clinical relevance in the DFIs. The availability of risk factors for them is very convenient for the choice of empirical treatment, especially, in moderate-severe infections.

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CONFLICT OF INTEREST

All authors have indicated they have no potential conflicts of interest to disclose.

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