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STAPHYLOCOCCUS AUREUS STRAINS ISOLATED FROM DIABETIC FOOT ULCERS. IDENTIFICATION OF THE ANTIBIOTIC RESISTANT PHENOTYPES

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Abstract

Background and aims*: Infected foot ulcer is one of the most feared complications of diabetes mellitus. Staphylococcus aureus is the most frequently isolated pathogen in diabetic foot infections. The aim of this study was to evaluate the prevalence of S. aureus strains involved in producing foot infections in diabetic patients and the antibiotic resistance pattern of these strains.* ***Material and methods****: The study included 33 S. aureus strains isolated from 55 diabetic foot ulcers. The subjects were selected from the 2465 patients with diabetes mellitus hospitalized in the Timişoara Diabetes Clinic, between 2011 and 2013. Germs' identification relied on cultural and biochemical characteristics. Final identification and antimicrobial testing were performed using the Vitek 2 (Bio Merieux France) automatic analyzer.* ***Results****: All the 55 samples collected from diabetic foot ulcers were positive. We isolated 64 bacterial strains (some samples were positive for 2 microorganisms). The most frequently isolated germ was S. aureus, in 33 samples (51.56%). All these S. aureus strains showed resistance to benzylpenicillin, while only 33.33% were methicillin-resistant (MRSA).* ***Conclusions****: The most frequently isolated germ in the wound secretions from diabetic foot ulcers was S. aureus. The highest percentage of antimicrobial resistance was recorded to benzylpenicillin and erythromycin.*

key words: *diabetic foot infections, S. aureus, antibiotic resistance*

Background and Aims

Diabetic foot ulcers are still a problem for the health care system and are majorly influencing quality of life. Treating these ulcers is mandatory in order to avoid progression of the infection and the need of amputations. Therefore, it is very important to identify the germ(s) involved and its (their) sensitivity to antibiotics. By using an aggressive and effective antibiotic therapy, these patients could have a favorable outcome.

Infected foot ulcer is one of the most feared complications of diabetes mellitus, leading to

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gangrene and need of amputations. The risk of a diabetic patient for developing a foot ulcer was estimated to be ~25% [1]. Management of the diabetic foot infections requires isolation and identification of the bacterial flora and an antibiotic therapy according to the antimicrobial susceptibility testing results [2]. It was showed that most diabetic foot infections are produced by plurimicrobial flora influenced by the bacterial flora of the lower limb, metabolic factors, foot hygiene and the use of antibiotics in the recent previous period [3].

Staphylococcus aureus (S. aureus) is the most frequently isolated pathogen in diabetic foot infections and almost 50% of *S. aureus* isolates are methicillin-resistant (MRSA) [4,5]. MRSA strains are resistant to methicillin and other semisynthetic antistaphylococcal penicillins (eg, oxacillin, dicloxacillin, and nafcillin). Frequently, this phenotype confers resistance to most beta-lactam antimicrobial agents, without including anti-MRSA cephalosporins (eg, ceftaroline and ceftobiprole) [6]. MRSA has emerged as a serious problem in patients with diabetic foot. Infections with MRSA may result in prolonged hospitalization and greater costs for the health system [7,8].

The aim of this study was to determine the prevalence of *S. aureus* strains involved in producing diabetic foot infections and the antibiotic resistance pattern of these strains in a group of patients from Timişoara, Romania.

Material and Methods

The study was conducted in the Diabetes Clinic of the Emergency Clinical County Hospital Timişoara during a two year period (2011-2013). In this interval, 2465 patients were admitted to the above mentioned department.

We isolated and included for analysis 64 bacterial strains from 55 samples of wound secretion collected from all the patients having foot ulcer(s). Collecting the samples from the diabetic foot ulcers and identification of germs were performed during patients' hospitalization. Specimens were collected according to sample specific collection protocols.

The collected wound secretions were inoculated on nonselective solid culture media (Columbia agar supplemented with 5% sheep blood) followed by incubation at 37°C for 24 hours, in 5-10% CO_2. Usual culture media were supplemented by selective and diagnostic media. For isolating staphylococci in the wound secretions, the solid Chapmann agar was used. Of the lactose containing media for enterobacteria, Mac Conkey was the most frequently used. Fungi were isolated on Sabouraud agar with chloramphenicol. Our study did not address anaerobic bacteria due to the lack of isolation conditions.

Germs' identification was generally done according to morphological, cultural, biochemical characteristics and pathogenicity tests. All the strains were identified using the Vitek 2 Compact automated system (bioMerieux).

The sensitivity to anti-infectious chemotherapeutic agents of the isolated germs was assessed by performing the MIC (Minimum Inhibitory Concentration) test, with automated reading and classification into resistance phenotypes by use of the Vitek 2 Compact analyzer, according to the CLSI (Clinical Laboratory and Standards Institute Inc.) [6]. Quality control strains used in the testing were *S. aureus* ATCC 43300 for MRSA strains and *S. aureus* ATCC 29213 for sensitive strains.

Statistical analysis. Data were collected and analyzed using SPSS v.17 (SPSS Inc. Chicago, IL) statistical software suite. Prevalence is expressed as percentages and represents the probability of observing a positive case in the studied population. The confidence interval for the prevalence was estimated using Wilson's method and for assessing the significance of

differences between prevalence in different groups Fisher's test was performed.

Results

Of the 55 wound secretions collected from the diabetic foot ulcers, all samples (100%) were positive, with 64 different bacterial strains. In 45 samples (81.81%), a single bacterial species was isolated, the most frequent one being *S. aureus* (53.33%). In pluri-microbial samples (that represented 18.19%), *S. aureus* was present in 90% of the cases, being frequently accompanied by the Enterobacteriaceae strains.

In diabetic foot ulcers in our study group, *S. aureus* was predominant with 33 strains, followed by Gram negative bacilli from the Enterobacteriaceae family: *Proteus mirabilis* – 9 strains, *Klebsiella pneumoniae* – 6 strains, *Escherichia coli* – 4 strains, *Enterobacter aerogenes* – 2 strains. Other Gram positive cocci were *Staphylococcus haemolyticus* (4 strains), *Staphylococcus epidermidis* (2 strains) and *Streptococcus agalactiae* (1 strain). Non fermentative Gram negative bacilli were represented by *Acinetobacter baumannii* (2 strains) and *Pseudomonas aeruginosa* (1 strain).

We tested the antibiotic resistance of the *S. aureus* isolates and the results obtained are presented in Table 1.

In Table 2 we present the antibiotic resistance phenotypes of *S. aureus* isolates as resulted in our samples.

Table 1. Resistance to antibiotics in *S. aureus* isolates.

Antibiotic	Susceptible		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Benzylpenicillin	–	–	–	–	33	100
Cefoxitin	22	66.66	–	–	11	33.33
Oxacillin	22	66.66	–	–	11	33.33
Imipenem	22	66.66	–	–	11	33.33
Ciprofloxacin	26	78.78	1	3.03	6	18.18
Moxifloxacin	27	81.81	–	–	6	18.18
Gentamicin	31	93.93	2	6.06	–	–
Streptomycin	31	93.93	–	–	2	6.06
Clindamycin	23	69.69	–	–	10	30.30
Erythromycin	10	30.30	1	3.03	22	66.66
Rifampicin	33	100	–	–	–	–
Tetracycline	27	81.81	–	–	6	18.18
Tigecycline	28	84.84	–	–	5	15.15
Linezolid	33	100	–	–	–	–
Fosfomycin	33	100	–	–	–	–
Fusidic acid	33	100	–	–	–	–
Vancomycin	33	100	–	–	–	–
Teicoplanin	33	100	–	–	–	–
Trimethoprim/ sulfamethoxazole	22	66.66	–	–	11	33.33

S. aureus showed absolute resistance to benzylpenicillin, while MRSA represented 33.33%. All of the MRSA strains also presented resistance phenotypes for other classes of antibiotics. Resistance to erythromycin occurred in 66.66% of the cases and to clindamycin in 30.3%. Most of the strains were sensitive to fluoroquinolones (around 80%), tetracyclines (around 80%) and aminoglycosides (around 90%), while all the strains were sensitive to rifampicin, linezolid, fosfomycin, fusidic acid and glycopeptides.

Table 2. Resistance phenotypes in *S. aureus* isolates.

Phenotypes	No.	%
PeniR	6	18.18
PeniR+TE	3	9.09
PeniR+SXT	2	6.06
PeniR+Q+SXT+MLSBc	1	3.03
PeniR+MLSBc	3	9.09
PeniR+MLSBi	4	12.12
PeniR+MLSBi+SXT	2	6.06
PeniR+MLSBc+SXT	1	3.03
MRSA+MLSBi	1	3.03
MRSA+MLSBc	1	3.03
MRSA+MLSBi+TE	1	3.03
MRSA+MLSBc+SXT	1	3.03
MRSA+MLSBc+Q+TE	2	6.06
MRSA+MLSBi+AG+Q+SXT	1	3.03
MRSA+MLSBc+AG+Q+SXT	1	3.03
MRSA+MLSBi+Q+TE+SXT	1	3.03
MRSA+MLSBi+SXT	1	3.03
MRSA+MLSBi+AG	1	3.03

Legend: PeniR- penicillin resistance, MRSA- methicillin-resistant *Staphylococcus aureus*, MLSBi- erythromycin resistance, MLSBc- erythromycin and clindamycin resistance, AG- aminoglycosides resistance, Q- fluoroquinolones resistance, SXT- trimethoprim-sulphamethoxazole resistance, Te- tetracyclines resistance

Discussions

In our study, all the collected specimens of wound secretions were positive. A limitation of this study is that the microbial surveillance was predominantly performed using swab samples, while tissue biopsies and secretion aspirates are considered the standard for diagnosing wound infections [9].

The most frequently isolated germ in our study group was *S. aureus* with 33 strains (51.56%). Similar results were obtained in other studies. For example, Mendes et al. also found *S. aureus* to be the most frequently isolated pathogen, present in 51% of the samples [10].

Ten specimens were positive for 2 concomitant microorganisms. From these 10 ulcer specimens, 9 involved strains of *S. aureus*, associated more frequently with *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* or *Enterobacter aerogenes*. Pluri-microbial flora involvement in the etiology of diabetic foot infections has been also observed in other various studies [3,11,12].

Regarding the antibiotic sensitivity pattern of the *S. aureus*, 11 strains (33.33%) belonged to the MRSA phenotype, which is also similar with the findings of Mendes et al., who recorded MRSA phenotype in 21.8% of the cases. [10] Other studies reported higher percentages (40-56%) of MRSA *S. aureus* strains [5,13,14].

Conclusions

In wound secretions from diabetic ulcers of the patients admitted to our hospital, the most frequently isolated germ was *S. aureus*. The highest percentage of antimicrobial resistance was recorded to benzylpenicillin and erythromycin. The prevalence of MRSA was 33.33%, all these strains being also resistant to other antibiotics. Most strains were sensitive to fluoroquinolones, tetracyclines and aminoglycosides and all of them to rifampicin, linezolid, fosfomycin, fusidic acid and glycopeptides.

We conclude that, before having the result of the microbiological analysis of a wound secretion collected from a diabetic foot ulcer, we could begin antibiotic therapy using one of the agents that *S. aureus* have shown to be sensitive to.

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