Prevalence of Staphylococcus aureus infection among diabetic foot patients in Sana'a city-Yemen

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Abstract

Diabetes mellitus is a progressive disease with chronic complications. Foot infections are a frequent complication for diabetic patients. The infection with Staphylococcus aureus in the diabetic foot accelerate the inflammatory process, endothelial injury and blood coagulation, ultimately lead to a faster death. The aim of this study was, firstly to determine the prevalence of S. aureus among diabetic foot patients, secondly to identify the predisposing factors associated with S. aureus infection and diabetic foot, and thirdly to determine the antibiotics that are effective against S. aureus isolates.

This was a case-finding study which included 93 of diabetic foot patients of whom 66 were males and 27 were females. Their age ranged from 16 to ≥ 76 years old, with a mean age of 60.5 years. These patients seeked medical attention for different diabetic foot infections at Al-Thawra General Hospital, Al-Gumhouri Teaching Hospital and Azal Specialized Hospital in Sana'a city, Yemen during the period, starting in December 2008 and ending in November 2009. A swab was collected from each infected diabetic foot patient and cultured for S. aureus using standard bacteriological procedures.

S. aureus was isolated from 56% of the total diabetic foot patients, among these isolates, MRSA represented 55.8% and VRSA 9.6%. Regarding the predisposing factors for the studied patients, hypertension and peripheral neuropathy represented 30.1% and 32.2%, but these results were not statistically significant in term of the patients and S.aureus infections with a relative risk for peripheral vascular disease patients who were one and half time more at risk than the others in contracting S.

aureus. As regards the antibiotics tested in this study, the efficacy of these antibiotics against S. aureus isolates is shown in the following order; imipenem (98.1%), cefepime (96.2%), vancomycin (90.4%), rifampicin (86.5%), ceftriaxone (73.1%), gentamicin (67.3%), clindamycin (67.3%), ciprofloxacin (65.4%), fusidic acid (63.5%), erythromycin (63.5%), tetracycline (55.8%), methicillin (44.2%), oxacillin (44.2%) and augmentin (32.7%). Finally, among all S. aureus isolates, 63.5% were multi-drug resistant and among all MRSA isolates, 69% were also multi-drug resistant.

Further studies are recommended to determine all the etiological agents that infect the diabetic foot other than S. aureus, especially for the rest governorates of the country and the periodical testing of more recent antibiotics for accurate susceptibility and therapy.

Introduction:

Staphylococcus aureus (S. aureus) is by far the most important human pathogen among the staphylococci. It is found in the external environment and in the anterior nares of 20-40% of adults. Other sites of colonization include intertriginous skin folds, the perineum, the axillae and the vagina. Although this organism is frequently a part of the normal human microflora, it can cause significant opportunistic infections under the appropriate conditions.[1]

S. aureus is an opportunistic pathogen that causes a broad range of human infections such as food poisoning, pneumonia, meningitis, skin infections, arthritis, osteomyelitis, endocarditis, and toxic shock syndrome.[2]

S. aureus has three features that make it distinct among most other clinically important bacteria. It can express a variety of virulence factors, it has the ability to develop and expand resistance to a broad spectrum of antimicrobial drug classes, and it is prominent in both hospital and community settings. These diverse features contribute to its pathogenicity and help to define the host-microbe interaction.[3, 4]

Foot infections are a major cause of morbidity in patients with diabetes worldwide. They occur in up to 15% of diabetic patients and account for 20% of all hospitalizations of diabetic patients.[5] Contributory factors include peripheral neuropathy, vascular disease, pedal deformities and local trauma and pressure. Diabetic foot infections (DFIs) are in turn an important risk factor for amputation, accounting for approximately two-thirds of lower-extremity amputations in diabetics [6]. Prompt institution of effective antimicrobial therapy for DFI should help reduce morbidity.[7] However, there is a lack of good-quality evidence of clinical efficacy and cost effectiveness to guide the choice of antimicrobial therapy. The choice of therapy is

becoming increasingly complex, driven both by increasing antibiotic resistance in the pathogens isolated from DFI [8] and an increasing number of new or forthcoming antibiotics licensed for treating skin and skin-structure infections (SSSIs).[9]

The infections with S. aureus in the diabetic foot accelerated the inflammatory process, endothelial injury, and blood coagulation, ultimately leading to a quicker death.[10]

Patients, Materials and Methods

This case-finding study included 93 diabetic foot patients of whom 66 were males and 27 were females. Their age ranged from 16 to ≥ 76 years old, with a mean age of 60.5 years. These seeked medical attention for different diabetic foot infections at Al-Thawra General Hospital, Al-Gumhouri Teaching Hospital and Azal Specialized Hospital in Sana'a city, Yemen, during a ~ 1 year period starting in December 2008 and ending in November 2009. A questionnaire for each patient was filled with the patient's personal and clinical information. This included the age, gender, occupation, diabetes duration, type of diabetes and the relevant clinical information regarding the diabetic foot infection. Peripheral sensory neuropathy was considered present if three or more sensory modalities were absent.[11] Peripheral vascular disease (PVD) was diagnosed if both foot pulses (dorsalis pedis and posterior tibialis) were absent on palpation from the ulcer-affected limb. Neuro-ischaemic ulceration was diagnosed if criteria for both PVD and sensory neuropathy were met.

All specimens collected by Dacron swabs from the site of diabetic foot infections were either put into transport medium for cultured later or directly cultured on mannitol salt agar (selective medium), then processed by using culture standardized methods and incubated aerobically at 35-37^{o}C for 24-48 hrs.[12] All S. aureus isolates were identified primarily by routine laboratory procedures. Gram-positive, catalase-positive colonies were tested for mannitol fermentation. clumping factor was detected by

using rabbit plasma. Organisms were confirmed as S. aureus by the tube coagulase test and the DNase test. S. aureus isolates were tested for antibiotics susceptibility by the Kirby-Bauer disc diffusion method using discs on Mueller-Hinton agar supplemented with 4% NaCl and incubated at 35 °C for 24 hrs. The antibiotic discs that were used in the antibiogram were augmenitin, cefepime, ceftriaxone, ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, imipenem, methicillin, oxacillin, rifampicin, tetracycline and vancomycin. Bacterial growth inhibition zones (mm) in diameter were measured according to the disc manufacturer's instructions.

The clinical and personal date in addition to the results of culture for each specimen were entered into a questionnaire and analyzed by the Epi Info, version 6, 2004, CDC. The significance of difference in proportion was analyzed by Pearson Chi-square (χ^2) which equal to or greater than 3.84, probability value (p) which equal to or less than 0.05 was considered as statistically significant.

Results:

Table 1: The number and percentage of S. aureus and other isolates in respect to total studied patients with diabetic foot infection

Culture results	*Total examined patients*	
	No.	*%*
S. aureus	*52*	*56.0*
*CONS**	*22*	*23.6*
No growth	*19*	*20.4*
Total	*93*	*100.0*

**CONS: Coagulase negative Staphylococcus*.

Table 1 shows the number and percentage of S. aureus and other isolates in respect to the total studied patients with diabetic foot infection. Accordingly, 52 (56%) of patients were S. aureus positive, 22 (23.6%) of patients were CONS positive and the remaining 19 (20.4) of patients had no growth. It clear from this that the infection with S. aureus was high in comparison with the other Staphylococcus spp which were remarkably low.

Table 2: The number and percentage of isolated S. aureus in respect to the gender of patients with diabetic foot infection

Gender	*Total*		*+ve S. aureus*		*RR*	*CI*	χ^2	*p*
	No.	*%*	*No.*	*%*				
Male	*66*	*71.0*	*37*	*56.1*	*1.1*	*0.7-1.6*	*0.05*	*0.82*
Female	*27*	*29.0*	*15*	*55.5*	*0.99*	*0.7-1.5*	*ND*	*0.96*
Total	*93*	*100.0*	*52*	*55.9*	-	-	-	-

Table 2 shows the number and percentage of isolated S. aureus in respect to the gender of patients with diabetic foot infection. Out of the 66 (71%) males, 37 (56.1%) were positive with S. aureus. Regarding the remaining 27 (29%) females, 15 (55.5%) were positive with S. aureus. These results were not statistically significant.

Table 3: The number and percentage of isolated S. aureus in respect to age of the patients with diabetic foot infection

Age [years]	*Total*		*+ve S. aureus*		*RR*	*CI*	χ^2	*p*
	No.	*%*	*No.*	*%*				
16-35	*6*	*6.5*	*4*	*66.6*	*1.2*	*0.7-2.2*	*0.3*	*0.6*
36-55	*25*	*26.9*	*16*	*64.0*	*1.2*	*0.84-1.75*	*0.91*	*0.34*
56-75	*53*	*57.0*	*26*	*49.0*	*0.75*	*0.5-1.1*	*2.35*	*0.12*
≥ 76	*9*	*9.6*	*6*	*66.6*	*1.2*	*0.74-2.01*	*0.5*	*0.49*
Total	*93*	*100*	*52*	*55.9*	-	-	-	-

Table 3 shows the number and percentage of isolated S. aureus in respect to age of patients with diabetic foot infection. Out of the 6 (6.5%) patients in the age group from 16 to 35 years, S. aureus was positive in 4 (66.6%) of these patients. Out of the 25 (26.9%) patients in the age group from 36 to 55 years, S. aureus was positive in 16 (64%) of these patients. Out of the 53 (57%) patients in the age group from 56 to 75 years, S. aureus was positive in 26 (49%) of these patients. Out of the 9 (9.6%) patients in the age group from ≥ 76 years, S. aureus was positive in 6 (66.6%) of these patients. These results were also not statistically significant.

Table 4: The number and percentage of isolated MRSA from the total positive S. aureus in respect to the gender of patients with diabetic foot infection

Gender	*Total S. aureus*		*MRSA**		*RR*	*CI*	χ^2	*p*
	No.	*%*	*No.*	*%*				
Male	*37*	*71.1*	*20*	*54.0*	*0.9*	*0.54-1.5*	*0.15*	*0.69*
Female	*15*	*28.9*	*9*	*60.0*	*1.1*	*0.7-1.85*	*0.15*	*0.7*
Total	*52*	*100.0*	*29*	*55.8*	-	-	-	-

**MRSA: Methicillin-resistant Staphylococcus aureus.*

Table 4 shows the number and percentage of isolated MRSA from the total positive S. aureus in respect to the gender of patients with diabetic foot infection. Out of the 37 (71.1%) males, 20 (54%) were positive with MRSA. Regarding the remaining 15 (28.9%) females, 9 (60%) were positive with MRSA. These results were also not statistically significant.

Table 5: The number and percentage of isolated VRSA from the total positive S. aureus in respect to the gender of patients with diabetic foot infection

Gender	*Total S. aureus*		*VRSA**		*RR*	*CI*	χ^2	*p*
	No.	*%*	*No.*	*%*				
Male	*37*	*71.1*	*3*	*8.1*	*0.6*	*0.11-3.3*	*0.34*	*0.56*
Female	*15*	*28.9*	*2*	*13.3*	*1.64*	*0.3-8.9*	*0.34*	*0.56*
Total	*52*	*100.0*	*5*	*9.6*	-	-	-	-

**VRSA: Vancomycin-resistant Staphylococcus aureus*

Table 5 shows the number and percentage of isolated VRSA from the total positive S. aureus in respect to the gender of patients with diabetic foot infection. Out of the 37 (71.1%) males, 3 (8.1%) were positive with VRSA. Regarding the remaining 15 (28.9%) females, 2 (13.3%) were positive with VRSA. These results were also not statistically significant. Females were nearly one and half time more at risk than males in contracting VRSA.

Table 6: The prevalence and relative risk of S. aureus infection in respect to the risk factors in patients with diabetic foot infection

Risk factors	*Total (n=93)*		*+ve S. aureus*		*RR*	*CI*	χ^2	*P*
	No.	*%*	*No.*	*%*				
Hypertension	*28*	*30.1*	*13*	*46.4*	*0.8*	*0.5-1.2*	*1.5*	*0.22*
Peripheral neuropathy	*30*	*32.2*	*16*	*60.0*	*0.93*	*0.63-1.4*	*0.12*	*0.72*
Peripheral vascular disease	*5*	*5.4*	*4*	*80.0*	*1.5*	*0.9-2.4*	*1.24*	*0.26*

Table 6 shows the prevalence and relative risk of S. aureus infection in respect to the risk factors in patients with diabetic foot infection. Out of the 28 (30.1%) patients with hypertension,

S. aureus was positive in 13 (46.4%) of patients. Out of the 30 (32.2%) patients with peripheral neuropathy, S. aureus was positive in 16 (60%) patients, while 5 (5.4%) of patients with peripheral vascular disease, S. aureus was positive in 4 (80%) patients. These results were also not statistically significant. Patients with peripheral vascular disease were one and half time more at risk than others in contracting S. aureus.

Table 7: The susceptibility patterns of S. aureus isolates towards the different commonly used antibiotics

Antibiotics	*Susceptibility test*					
	Sensitive		*Intermediate*		*Resistant*	
	No.	*%*	*No.*	*%*	*No.*	*%*
Augmentin (30µg)	*17*	*32.7*	*-*	*-*	*35*	*67.3*
Methicillin (5µg)	*17*	*32.7*	*6*	*11.5*	*29*	*55.8*
Oxacillin (1µg)	*17*	*32.7*	*6*	*11.5*	*29*	*55.8*
Tetracycline (30µg)	*21*	*40.4*	*8*	*15.4*	*23*	*44.2*
Erythromycin (15µg)	*22*	*42.3*	*11*	*21.1*	*19*	*36.6*
Fusidic acid (10µg)	*30*	*57.7*	*3*	*5.8*	*19*	*36.5*
Ciprofloxacin (5µg)	*26*	*50.0*	*8*	*15.4*	*18*	*34.6*
Clindamycin (2µg)	*27*	*51.9*	*8*	*15.4*	*17*	*32.7*
Gentamicin (10µg)	*33*	*63.5*	*2*	*3.8*	*17*	*32.7*
Ceftriaxone (30µg)	*34*	*65.4*	*4*	*7.7*	*14*	*26.9*
Rifampicin (5µg)	*41*	*78.8*	*4*	*7.7*	*7*	*13.5*
Vancomycin (30µg)	*47*	*90.4*	*-*	*-*	*5*	*9.6*
Cefepime (30µg)	*40*	*77.0*	*10*	*19.2*	*2*	*3.8*
Imipenem (10µg)	*51*	*98.1*	*0*	*0.0*	*1*	*1.9*

Table 7 shows the susceptibility patterns of S. aureus isolates towards the different commonly used antibiotics. The percentages of antibiotics to which isolated S. aureus was

resistant is shown in the following order; augmentin (67.3%), methicillin (55.8%), oxacillin (55.8%), tetracycline (44.2%), erythromycin (36.5%), fusidic acid (36.5%), ciprofloxacin (34.6%), clindamycin (32.7%), gentamicin (32.7%), ceftriaxone (26.9%), rifampicin (13.5%), vancomycin (9.6%), cefepime (3.8%) and imipenem (1.9%).

Table 8: The multi-drug resistance profile of isolated S. aureus from diabetic foot patients

No. of antibiotics to which S. aureus was resistant	*MDR* S. aureus n=33*	
	No.	*%*
11	*4*	*12.1*
10	*4*	*12.1*
9	*3*	*9.1*
8	*2*	*6.1*
7	*4*	*12.1*
6	*2*	*6.1*
5	*1*	*3.0*
4	*2*	*6.1*
3	*11*	*33.3*

**MDR: Multi-drug resistant.*

Table 8 shows the multi-drug resistant profile of isolated S. aureus from diabetic foot patients. Out of the 52 isolated S. aureus, MDR S. aureus was positive in 33 (63.5%). The number of antibiotics to which isolated S. aureus was resistant is shown in the following order: 4 (12.1%) isolates were resistant to 11 different antibiotics, 4 (12.1%) isolates were resistant to 10 different antibiotics, 3 (9.1%) isolates were resistant to 9 different antibiotics, 2 (6.1%) isolates were resistant to 8 different antibiotics, 4 (12.1%) isolates were resistant to 7 different antibiotics, 2 (6.1%) isolates were resistant to 6 different antibiotics, 1 (3.0%) isolate was resistant to 5 different antibiotics, 2 (6.1%) isolates were resistant to 4 different

antibiotics, 11 (33.3%) isolates were resistant to only 3 different antibiotics.

Discussion:

S. aureus is a common cause of diabetic foot ulcers (DFUs) infection. S. aureus, either alone or as a component of mixed infections, is the most important pathogen in a diabetic foot infection.[13] Infection with methicillin-resistant Staphylococcus aureus (MRSA) is an increasing problem in both hospital and the community.[14,15] MRSA is commonly grown from foot ulcer swabs of diabetic patients.[16]

This is the first study conducted in Yemen to report about the prevalence of S. aureus infection among diabetic foot patients.

In the present study the prevalence of S. aureus isolated from diabetic foot patients was 56%, this result was higher than that documented in Kuwait (44.2%), UK (42%) and Iran (26.2%),[17,18,19] but consistent to that documented by Slater in 2004, who found that the predominance of S. aureus in 50% of diabetic foot patients.[20]

In addition, the prevalence of coagulase negative Staphylococcus (CONS) isolates in this study was 23.6%, this result was higher than that documented in Spain (17.2%) [21] and consistent to that stated in India which was 25.9%.[22] The high percentage of Staphylococcus species in the present study may be attributed to the majority of diabetic foot infections (DFIs) that were superficial. The same finding was documented in Saudi Arabia, where the majority of DFIs is superficial and are frequently colonized by aerobic gram-positive bacteria.[23]

The cultures which showed no growth in this study were 20.7%, this result was higher than that reported in India (6.4%),[22] but lower than that reported in Spain (30%).[21] There is no clear explanation for the apparent differences in the microbiological

findings between developed and developing countries. The possible reasons may include patients presenting to medical services later in developing countries or the differences in the pattern of wound exposure to microorganisms or may referred to differences in microbiological diagnostic techniques.

Regarding the gender, there was no significant association in the prevalence of isolated S. aureus from DFIs, despite the incidence of diabetic foot infections among males was 71% versus 29% in females with a ratio of 2.4:1. This finding was similar to that reported in UK,[19] and other studies that underscored male preponderance for this condition in general. This may indicate a higher level of activity among males compared to females.

Although there was no statistical association between S. aureus and the different age groups, the lowest positivity for S. aureus was found in the age group (56-75) years old. This variation may be attributed to the large number of patients included in this group who are usually suffering from diabetes mellitus more than other age groups.

In this study, there is no significant association between the MRSA isolates and gender. But the prevalence of MRSA among diabetic foot patients was 31.1% from the total studied patients and 55.8% from the total S. aureus isolates, the finding of MRSA in relation to the patients was higher than that documented in UK (15%), France (16%), Kuwait (5.9%), India (10.3%) and UK (19%),[16,17,19, 22,24] but similar to that stated in UK (30%) and Egypt (31.3%).[25,26]

These variations in different countries can be explained by the different patient populations, hospital care practices, infection control activities, time of study and the biological characteristics of the S. aureus strains.

Regarding the prevalence of VRSA isolates among diabetic foot patients which yielded a percentage of 5.4% out of the total

studied patients and 9.6% out of the total S. aureus isolates. the finding of VRSA in relation to isolated S. aureus was higher than that documented in Kuwait and Malaysia which was zero for each,[17,27] but lower than that documented in Iran (63%).[18] Chronic ulcers and vancomycin use place one at risk for emerging VRSA.[28] From the present results, VRSA was 8.1% in males versus 13.3% in females, this variation may be due to the less number of females enrolled in the present study than males.

In this study, as regards the factors that contribute for diabetic foot infections, 30.1% of patients had hypertension, these results were lower than that reported in Kuwait (45.3%) and Nigeria (81.5%).[17,29] In addition, 32.2% of patients had peripheral neuropathy, these results were lower than that reported in UK (55%),[19] but higher than that documented in Spain (26%) and India (27.2%).[21,22] In this study, patients with peripheral vascular disease were 5.4%, this result was lower than that reported in Spain (27%) and India (10.3%).[21,22] The relative risk among patients with peripheral vascular disease was one and half time more at risk than other diabetic foot patients in contracting S. aureus. This may referred to the fact that these patients have impaired microvascular circulation which limits the access of phagocytic cells to the infected area and results in a poor concentration of antibiotics in the infected tissues. There was no literature found that correlated these factors with S. aureus infection.

The antibiogram results in this study suggested that the antibiotics that remain highly sensitive against S. aureus were; imipenem (98.1%), although most other studies used it for the susceptibility testing in gram-negative bacteria only, followed by cefepime (96.2%) which had a high activity against MRSA, this finding differs from a study conducted in Kuwait, which found that the fourth generation of cephalosporin; cefepime lack an adequate activity against MRSA.[17] The highest resistance of antibiotics used in the present study was found in augmentin

(67.3%), this result was higher than that documented in Kuwait 5.9%.[17] The resistant to erythromycin in this study was 36.5%, this finding was higher than that documented in Malaysia (16%),[27] but consistent to that documented in Kuwait (38.2%).[17] In addition, the resistant to gentamycin and fusidic acid in the present study were 32.7% and 36.5%, these results were higher than that documented in Kuwait which were 10% and 11.8% and in Malaysia with percentages of 18% and 7%.[17,27] But the resistant to tetracycline in this study was 44.2%, this result was lower than that documented in Kuwait 67.6%.[17] Moreover the resistant to clindamycin and ceftriaxone in this study was 32.7% and 26.6%, these results were lower than that reported in Iran which were 54 % and 81%.[18] Finally, resistant to oxacillin and rifampicin in this finding were 55.8% and 13.5%, these results were higher than that documented in Malaysia which were 16% and zero.[27]

The antibiotic profile of isolated S. aureus in this study revealed that the 63.5% of these isolates were multi-drug resistant. This result was lower than that documented in Nigeria,[29] which found that all S. aureus isolates from diabetic foot patients were 100% multi-drug resistant. Out of the 29 MRSA isolates, 20 (69%) were multi-drug resistant. Several reasons may account for the high rate of resistance among S. aureus in this study, these include; misuse of antibiotics, prescription of antibiotics without adequate knowledge about infectious diseases and proper antimicrobial usage. Moreover some patients in this study may self-medicated themselves or used local herbs for treatment, thereby tampering with their ulcers before admitting to the hospital which may also explain in part the multi-drug resistant nature of these S. aureus isolates. All isolated VRSA had also been resistant to methicillin and oxacillin. Severin and co-workers in 2004 investigated the mechanism of expression of high-level vancomycin resistance using an oxacillin-resistant S. aureus strain carrying the vanA gene complex and the inactivated mecA. They reported that the

key penicillin-binding protein essential for vancomycin resistance and for the altered cell wall composition characteristic of VRSA is PBP2. They also concluded that although mecA is essential for methicillin and oxacillin resistance, it is not involved in the expression of vancomycin resistance.[30]

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