ORIGINAL ARTICLE

Antimicrobial susceptibility of bacterial and fungal infections among infected diabetic patients

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Abstract

Objective: To determine the antimicrobial susceptibility of the most common bacterial and fungal infections among infected diabetic patients.

Methods: This study was conducted at the Umm Al-Qura University, Makkah, Saudi Arabia, from June 2011 to June 2012, and comprised specimens collected from diabetics. Antibiotic susceptibility test using disc diffusion method was performed for bacterial isolates, and antifungal susceptibility test using colorimetric method for candida isolates.

Results: Of the 138 specimens, antibacterial susceptibility test was performed for 129(93.5%) bacterial isolates while antifungal resistance test was performed for 9(6.5%) candida isolates. Of the bacterial isolates, 27(20.9%) were escherichia coli, 26(20.1%) staphylococcus aureus and 15(11.6%) were pseudomonas aeruginosa isolates.

All Escherichia coli and staphylococcus aureus were susceptible to amikacin and vancomycin (100% each). Moreover, all candida species were susceptible to amphotericin B, econazole, fluconazole, ketoconazole and nystatin (100% each).

Conclusion: The most effective antibiotics for bacterial infections among diabetic patients were vancomycin for gram-positive bacteria, amikacin for gram-negative bacteria and for bacteria isolated from diabetic patients with foot infections.

Keywords: Diabetic patients, Antibiotic susceptibility test, Antifungal susceptibility test. (JPMA 66: 1291; 2016)

Introduction

Diabetes is a common metabolic disorder that affects more than 180 million people worldwide and more than 24% of population in Saudi Arabia.[1] The World Health Organisation (WHO) has reported that Saudi Arabia ranks the second highest in the Middle East and seventh in the world for the rate of diabetes. It is estimated that around 7 million of the population are diabetic and almost around 3 million have pre-diabetes.[2]

Diabetics are more susceptible to various bacterial and fungal infections than non-diabetics.[3,4] This may be attributable to many risk factors resulting from diabetes such as vascular abnormalities,[5] nephropathy,[6] neuropathy,[7] delayed wound healing and immune depression.[8,9]

In a previous study performed by us,[10] the detected infections among 139 infected diabetic patients examined were 92.8% bacterial infections and 7.2% fungal infections, and included diabetic foot infections (40.3%), urinary tract infections (UTIs) (20.1%), respiratory tract infections (RTIs) (16.5%), skin infections (10.8%), septicaemia (10.1%), genital tract infections ((GTIs) 1.4%) and eye infection (0.7%). The most common isolated bacteria from infected diabetic patients in that study were Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus), while Candida species were the most common fungi isolated.

The treatment of any infection in diabetics is more difficult than non-diabetics, especially when there is poor glycaemic control.[11] The current study was planned to determine the antimicrobial susceptibility of the most common bacterial and fungal infections among infected diabetics.

Materials and Methods

This study was conducted at the Umm Al-Qura University, Makkah, Saudi Arabia, from June 2011 to June 2012, and comprised specimens collected from diabetics at different hospitals in Makkah and Jeddah.

All specimens were cultured in suitable media, incubated and identified manually according to growth characteristics, colonial morphology, gram stain and proper biochemical tests and confirmed using VITEK II machine according to manufacturer's instructions.

Antibacterial susceptibility test was performed for every isolated bacteria using disc diffusion method described

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by Bauer et al.[12] The antibiotics that were used against gram-positive bacteria were (20 µg of amoxicillin + 10 µg of clavulanate), ceftazidime (30µg), cephalothin (30µg), clindamycin (2µg), erythromycin (15µg), gentamycin (10µg), oxacillin (1µg), penicillin (10µg) and vancomycin (30µg).

Against gram-negative bacteria, the following antibiotics were used: amikacin (30µg), (20µg of amoxicillin + 10µg of clavulanate), cefotaxime (30µg), ceftazidime (30µg), cephalothin (30µg), chloramphenicol (30µg), ciprofloxacin (5µg), clindamycin (2µg), piperacillin (100µg) and tobramycin (10µg).

For bacteria isolated from diabetic foot infection, the following antibiotics were used against the causative agents: amikacin (30µg), (20µg of amoxicillin + 10µg of clavulanate), cefotaxime (30µg), ceftazidime (30µg), ciprofloxacin (5µg), erythromycin (15µg), gentamycin (10µg), oxacillin (1µg), piperacillin (100µg) and vancomycin (30µg).

The following antibiotics were used for bacteria isolated from urine cultures; amikacin (30µg), (20µg of amoxicillin + 10µg of clavulanate), cefotaxime (30µg), cephalothin (30µg), gentamycin (10µg), nalidixic acid (30µg), nitrofurantion (300µg), norfloxacin (10µg), sulfamethoxazole (100µg) and vancomycin (30µg) (Bioanalyse, Turkey).

Antifungal resistance test was performed for every isolated fungi using colorimetric method. This test was used against candida using Candifast ES Twin kit (Elitech France SAS, France) according to manufacturer's instructions. In this test, seven antifungal agents were used, including amphotericin B (4µg/ml), nystatine (200 units/ml), flucytosine (35µg/ml), econazole (16µg/ml), ketoconazole (16µg/ml), miconazole (16µg/ml) and fluconazole (16µg/ml).

Results

Of the 138 specimens, antibacterial susceptibility test was performed for 129(93.5%) bacterial isolates, while

Table-1: Antibiotics susceptibility testing for 21 Gram-positive bacteria and 31 Gram-negative bacteria isolated from infected diabetic patients.

21 Gram-positive bacteria			31 Gram-negative		
Antibiotics	**S***	**R****	**Antibiotics**	**S**	**R**
Amoxicillin-clavulanate	7 (33.3%)	14 (66.7%)	Amikacin	19 (61.3%)	12 (38.7%)
Ceftazidime	0	21 (100%)	Amoxicillin-clavulanate	1 (3.2%)	30 (96.8%)
Cephalothin	9 (42.9%)	12 (57.1%)	Cefotaxime	3 (9.7%)	28 (90.3%)
Clindamycin	6 (28.6%)	15 (71.4%)	Ceftazidime	9 (29%)	22 (71%)
Erythromycin	5 (23.8%)	16 (76.2%)	Cephalothin	0	31 (100%)
Gentamycin	6 (28.6%)	15 (71.4%)	Chloramphenicol	8 (25.8%)	23 (74.2%)
Oxacillin	8 (38.1%)	13 (61.9%)	Ciprofloxacin	11 (35.5%)	20 (64.5%)
Penicillin	0	21 (100%)	Clindamycin	0	31 (100%)
Vancomycin	21 (95.2%)	1 (4.8%)	Piperacillin	6 (19.4%)	25 (80.6%)

*S = Susceptible **R= Resistant

Table-2: Antibiotics susceptibility testing for 55 bacteria isolated from diabetic patients with foot infections and 22 bacteria isolated from diabetic patients with UTI.

55 bacteria isolated from diabetic patients with foot infections			22 bacteria isolated from diabetic patients with UTI		
Antibiotics	**S**	**R**	**Antibiotics**	**S**	**R**
Amikacin	45 (81.8%)	10 (18.2%)	Amikacin	14 (63.6%)	8 (36.4%)
Amoxicillin-clavulanate	25 (45.5%)	30 (54.5%)	Amoxicillin-clavulanate	8 (36.4%)	14 (63.6%)
Cefotaxime	27 (49.1%)	28 (50.9%)	Cefotaxime	4 (18.1%)	18 (81.8%)
Ceftazidime	25 (45.5%)	30 (54.5%)	Cephalothin	4 (18.1%)	18 (81.8%)
Ciprofloxacin	26 (47.7%)	29 (52.7%)	Gentamycin	10 (45.5%)	12 (54.5%)
Erythromycin	12 (21.8%)	43 (78.2%)	Nalidixic acid	0	22 (100%)
Gentamycin	36 (65.5%)	19 (34.5%)	Nitrofurantion	16 (72.7%)	6 (27.3%)
Oxacillin	7 (12.7 %)	48 (87.3 %)	Norfloxacin	8 (36.8%)	14 (63.2%)
Piperacillin	24 (43.6%)	31 (56.4%)	Sulfamethoxazole	6 (27.3%)	16 (72.7%)
Vancomycin	16 (29.1%)	39 (70.9%)	Vancomycin	4 (18.1%)	18 (81.8%)

*S = Susceptible **R= Resistant
UTI: Urinary tract infection.

Table-3: Antibiotics susceptibility testing for 18 E. coli isolated from diabetic patients with infections other than UTI, 9 E. coli isolated from diabetic patients with UTI. And 26 S. aureus isolated from infected diabetic patients.

18 E. coli isolated from diabetic patients with infections other than UTI			9 E. coli isolated from diabetic patients with UTI			26 S. aureus		
Antibiotics	**S**	**R**	**Antibiotics**	**S**	**R**	**Antibiotics**	**S**	**R**
Amikacin	18 (100%)	0	Amikacin	9 (100%)	0	Amikacin	12 (46.2%)	14 (53.8%)
Amoxicillin-clavulanate	7 (38.9%)	11 (61.1%)	Amoxicillin-clavulanate	4 (44.4%)	5 (55.6%)	Amoxicillin-clavulanate	13 (50%)	13 (50%)
Cefotaxime	9 (50%)	9 (50%)	Cefotaxime	3 (33.3%)	6 (66.7%)	Cefotaxime	10 (38.5%)	16 (61.5%)
Ceftazidime	9 (50%)	9 (50%)	Cephalothin	3 (33.3%)	6 (66.7%)	Ceftazidime	0	26 (100%)
Chloramphenicol	16 (88.9%)	2 (11.1%)	Gentamycin	6 (66.7%)	3 (33.3%)	Cephalothin	12 (46.2%)	14 (53.8%)
Ciprofloxacin	5 (27.8%)	13 (72.2%)	Nalidixic acid	0	9 (100%)	Ciprofloxacin	11 (42.3%)	15 (57.7%)
Erythromycin	0	18 (100%)	Nitrofurantion	8 (88.9%)	1 (11.1%)	Clindamycin	11 (42.3%)	15 (57.7%)
Gentamycin	13 (72.2%)	5 (27.8%)	Norfloxacin	3 (33.3%)	6 (66.7%)	Erythromycin	12 (46.2%)	14 (53.8%)
Piperacillin	4 (22.2%)	14 (77.8%)	Sulfamethoxazole	4 (44.4%)	5 (55.6%)	Gentamycin	12 (46.2%)	14 (53.8%)
Tobramycin	9 (50%)	9 (50%)				Oxacillin	11 (42.3%)	15 (57.7%)
						Penicillin	0	26 (100%)
						Piperacillin	6 (23.1%)	20 (76.9%)

*S = Susceptible **R= Resistant
UTI: Urinary tract infection
E. coli: Escherichia coli. S. aureus: Staphylococcus aureus.

antifungal resistance test was performed for 9(6.5%) candida isolates.

Of the bacterial isolates, 21(16.3%) were gram-positive bacteria, 31(24%) gram-negative, 55(42.6%) bacteria were isolated from diabetics with foot infections, whereas 22(17%) were isolated from diabetics with UTI.

Of the gram-positive bacteria, 21(95.45%) were susceptible to vancomycin; only enterococcus faecium showed resistance. All of the gram-positive bacteria showed resistance to penicillin and ceftazidime (Table-1).

Of the gram-negative bacteria, 19(61.3%) were susceptible to amikacin, whereas all of them were resistant to cephalothin and clindamycin.

Of the bacteria isolated from diabetic foot infections, 45(81.8%) were susceptible to amikacin and 36(65.5%) to gentamycin, while 48(87.2%) showed resistance to oxacillin and 43(78.2%) to erythromycin (Table-2).

Of the bacteria isolated from diabetics suffering from UTI, 16(72.7%) were susceptible to nitrofurantion and 14(63.6%) to amikacin. All of these bacteria showed resistance to nalidixic acid.

Of all, there were 27(20.9%) E. coli, 26(20.1%) S. aureus and 15(11.6%) P. aeruginosa isolates.

Of the E. coli isolates, 18(66.7%) were isolated from diabetics with infections other than UTI, whereas 9(33.3%) were from those infected with UTI. Of the former, all were susceptible to amikacin and resistant to erythromycin. Besides, 16(88.9%) were susceptible to chloramphenicol and 14(77.8%) were resistant to piperacillin. Of the latter, all 9(100%) were susceptible to amikacin and resistant to nalidixic acid, while 8(88.9%) were susceptible to nitrofurantion (Table-3).

Of the S. aureus isolates, all 26(100%) were susceptible to vancomycin and resistant to penicillin and ceftazidime. Moreover, 26(76.9%) were susceptible to piperacillin.

Of the P. aeruginosa isolates,14(93.3%) showed susceptibility to amikacin and tobramycin each, and all of them were resistant to Amoxicillin-clavulanate, cefotaxime and erythromycin. In addition, 11(73.3%), 10(66.7%) and 9(60%) isolates showed susceptibility to ciprofloxacin, chloramphenicol and ceftazidime, respectively. Besides, 10(66.7%) and 8(53.3%) isolates showed resistance to gentamycin and piperacillin, respectively.

Of the candida isolates tested for antifungal resistance, 4(44.4%) were C. albicans and 5(55.6%) were candida species. All C. albicans isolates were susceptible to amphotericin B, nystatine and fluconazole, 2(50%) to flucytosine and ketoconazole, and 1(25%) to miconazole. All of them were resistant to econazole.

For candida species, all were susceptible to amphotericin B, nystatin, fluconazole, flucytosine, ketoconazole and econazole, while 3(60%) were resistant to miconazole.

Discussion

Antibiotics were one of the most important scientific successes in the 20th century. However, this success faced

some problems due to emergence of antibiotic-resistant bacteria.[13] Antibiotic resistance of bacteria is a global concern for healthcare providers. The irregular and widespread usage of antibiotics was the principal cause of appearance of new antibiotic-resistant strains of bacteria, particularly in hospitals.[14]

Generally, the effectiveness of antimicrobial agents is influenced by various factors including diabetes-related factors such as glycaemic control, nephropathy and simultaneous complications. Other factors may include infection severity, causative organism property, selection of specific agents and their appropriate dose.[15]

Gram-positive bacteria usually respond rapidly to many antibiotics such as augmentin, cephalosporin, erythromycin, clindamycin, vancomycin and beta-lactam antibiotics (penicillin, amoxicillin, and oxacillin).[16]

In this study, gram-positive bacteria isolated from diabetics were tested against different antibiotics. Nearly all gram-positive bacteria were susceptible to vancomycin, except one isolate of enterococcus faecium that was classified as vancomycin-resistant enterococcus (VRE). Conversely, all gram-positive bacteria were resistant to penicillin and ceftazidime, while 76.2%, 71.4% and 71.4% were resistant to erythromycin, clindamycin and gentamycin, respectively. regarding S. aureus, which was the most common gram-positive bacteria, 100% isolates were susceptible to vancomycin and 50% to augmentin. This was in agreement with Buxton's study which reported that vancomycin is the antibiotic of choice for S. aureus infection.[17]

In the current study, gram-negative bacteria were tested against varieties of antibiotics. Most of them were sensitive to amikacin (61.3%), while 100% of them were totally resistant to cephalothin and clindamycin and 90.3% were resistant to cefotaxime. E. coli was the most common isolated organism from diabetic patients in general and as gram-negative bacteria in particular. All E. coli isolates, other than those responsible for UTI, were highly susceptible to amikacin (100%) while 88.2% and 72.2% were susceptible to chloramphenicol and gentamycin, respectively. However, 100% of them were resistant to erythromycin. Findings of this study were in agreement with findings from several studies[18-20] which reported that E. coli is considered the most common isolates in many gram-negative bacterial infections, and it is mostly susceptible to amikacin. In the present study, too, P.aeruginosa was found to be the third most common organism isolated from diabetic patients. They were mostly susceptible (93.3%) to amikacin and tobramycin. However, Amoxicillin-clavulanate, cefotaxime and erythromycin were fully resisted by all isolates of P. aeruginosa in this study.

A specific group of antibiotics was selected for bacteria isolated from diabetic patients with foot infection, the most common infection encountered in this population. Since most of bacteria isolated from diabetic foot patients in this study were gram-negative bacteria (particularly E. coli), amikacin was the antibiotic of choice as 81.8% of bacteria isolated were susceptible to it. This was in agreement with Sekhar et al.[21] who reported that all gram-negative isolates from diabetic patients with foot infection, including extended spectrum beta lactamase producing strains of Proteus mirabilis and klebsiellaoxytoca except acinetobacter, were highly sensitive to Amikacin. However, 87.3% and 70.9% of bacteria causing foot infections in this study were resistant to oxacillin and vancomycin, respectively.

The second most common infection found in this study was UTI. Most bacteria causing UTI were susceptible to nitrofurantion (72.7%), followed by amikacin (63.6%). This was in accordance to Falagas et al.[18] findings who reported that UTIs are mostly treated with amikacin and nitrofurantion. However, 100%, 81.8% and 81.8% of bacteria causing UTI in our study were resistant to nalidixic acid, cefotaxime and cephalothin, respectively. E. coli was the most common organism isolated from patients with UTI. All these E. coli isolates were susceptible to amikacin (100%) and 88.9% of them were susceptible to nitrofurantion, while 100% of them were resistant to nalidixic acid. Mathai et al.[19] and Vikas et al.[20] recommended that amikacin and nitrofurantion are a good choice for UTI treatment.

In the present study, all C. albicans isolates from diabetic patients were highly susceptible to amphotericin B, fluconazole and nystatin (100%) while 100% of them were resistant to econazole and 75% were resistant to miconazole. For candida species, 100% were susceptible to amphotericin B, fluconazole, econazole, nystatin and ketoconazole, while 60% of them were resistant to miconazole. This was in agreement with findings of Goswami et al.[22] Abdulrazak et al.[23] and Lattif[24] who reported that candida, especially non-albicans species, are mostly susceptible to amphotericin B, fluconazole, econazole and nystatin.

It is hoped that the results obtained from this study will be of great benefits to physicians who frequently deal with diabetic patients in their proper selection of empirical therapy and to diabetic patients themselves who are highly susceptible to infections to take care of themselves.

In addition, we recommend extending this project to cover different regions and cities of Saudi Arabia to give a broader picture about the situation of bacterial and fungal infections among diabetic patients and their antimicrobial susceptibility testing.

Conclusion

Most effective antibiotics for bacterial infections among diabetic patients were vancomycin for gram-positive bacteria, amikacin for gram-negative bacteria and for bacteria isolated from diabetic patients with foot infections, and nitrofurantion for bacteria isolated from diabetic patients with UTI. E. coli and S. aureus were highly susceptible to amikacin and vancomycin, respectively. The most effective antifungal agents for candida infections among diabetic patients were amphotericin B, fluconazole and nystatin.

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