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Resistance profile of bacteria species isolated from wound samples of diabetic and non-diabetic patients in a tertiary hospital in Abakaliki, Nigeria

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Abstract

Diabetes is a medical condition that predisposes the sufferers to several secondary (bacterial-related) infections. This study determined the resistance profile of bacteria species isolated from the wound samples of diabetic and non-diabetic patients with a view to update their antibiogram and provide reference point to guide therapy. Swab samples from the wounds of diabetic male patients (n=32) and diabetic female patients (n=41) patients were analyzed using standard microbiological techniques, as well as selective culture media for the isolation and characterization of some priority antibiotic resistant bacteria. Six bacteria species comprising *Proteus specie, Escherichia coli, Pseudomonas aeruginosa, Salmonella* specie, *Shigella* species and *Staphylococcus aureus* were bacteriologically recovered and characterized for the susceptibility studies. Corresponding swab samples from the wounds of non-diabetic patients were also included in this study. Antibiotic susceptibility studies were undertaken using the modified Kirby-Bauer disk diffusion technique while the multiple antibiotic resistance profile of the isolated bacteria was determined using the multiple antibiotic resistance index (MARI). *E. coli, Proteus* species, *Shigella* species and *P. aeruginosa* from diabetic and non-diabetic patients showed reduced susceptibility to ofloxacin (100%), ceftazidime (100%), amoxicillin-clavulanic acid, AMC (100%) and aztreonam (100%). *Salmonella* species from diabetic and non-diabetic patients showed reduced susceptibility to ofloxacin (100%), ceftazidime (100%), AMC (100%), and aztreonam (100%). For ofloxacin, the *Salmonella* species from non-diabetic patients showed a 50% resistance to ofloxacin. *S. aureus* was isolated from only diabetic patients and found to show reduced susceptibility to ofloxacin (75%), clindamycin (100%), chloramphenicol (83%), amikacin (100%) and lincomycin (70%). On average, the MARI of the isolated bacteria (MARI=0.7) showed multiple resistance profile to at least 7 antibiotics from different classes. Prompt detection and reporting of the antibiogram of bacteria from clinical cases including diabetes is important to guide therapy in order to mitigate the emergence and spread of drug resistant strains of pathogenic bacteria, as well as improve the prognosis of the affected patients.

Keywords: Antibiotic resistance; Pathogenic bacteria; Diabetes; Sensitivity studies

Introduction

Antimicrobial resistance (AMR) is a global public health crisis that makes it difficult to select optimal therapy for the affected individuals owing to the growing cases of drug resistant bacteria in both the hospital and community settings. It is a current health menace that strikes at the core of infectious disease control in the hospital environment, and has potential to affect every aspect of healthcare systems especially in the developing economies (Ejikeugwu *et al*., 2022a). AMR is a significant health menace of this century and impacts developing healthcare systems – which is why it is important to detect and report it even in wound-inflicted cases (Ejikeugwu *et al*., 2022b). A wound is a type of injury which happens relatively quickly in which skin is torn, cut or punctured, or where blunt force trauma causes a contusion or a closed wound (Adegoke *et al.,* 2010). In pathology, wound refers to a sharp injury which damages the dermis of the skin and therefore compromises its protective function. The damage to the dermis of the skin which culminates to a wound can result to microbial contamination which may predispose the individual to further clinical case. Infection also occurs when virulence factors produced by the invading microorganisms overwhelm the host natural resistance (Bowler *et al.,* 2011). Wound infection is characterized by the presence of pus in lesions with pyrexia, pain and induration. The unbroken skin is the first line of defense and a barrier against microbial invasion which serves as host to a variable number of transient or contaminating bacteria (Stephan and Landis, 2008). Although its low surface pH, sebaceous fluid, and fatty acids produced, inhibits the colonization and growth of pathogenic organisms, exposure of subcutaneous tissue following loss of skin integrity caused by a wound provides a moist, warm, and nutritious environment that is conducive for microbial colonization and proliferation. The presence of

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pathogenic organisms in wound without local or systemic tissue involvement may not result in infection (Sule *et al.,* 2012). The infecting or invading microorganisms can cause destruction and tissue damage if left untreated (AWMA, 2011). Bacterial wound infections are important because they can slow down the healing process, and thus lead to wound breakdown, prolonged hospital stay and increase in the cost of treatment. According to previous studies, high index of wound contamination with bacteria are the third most frequent nosocomial infections around the globe (Ducel *et al.,* 2010). Diabetic patients are at increased risk of wound infection during minor or major surgery due to the development of inflammatory processes that culminates in increased risk of secondary infection and impaired wound healing. Septic operation in tropical countries is seen to have contributed to high rate of mortality especially in diabetes and non-cardio logical critically ill patients, with as many as 20 million cases of sepsis and a mortality rate of around 35% reported worldwide annually (Mike *et al.,* 2015). Diabetes and surgery increase the risk of infection and impaired wound healing in people who are predisposed to wound infections. Diabetes mellitus and invasive procedures (surgery) both play a significant role in alteration of host defense. Furthermore, post-operative infection associated with diabetes are the second most common healthcare associated problems resulting in prolonged hospitalization, higher cost of medical care with increase morbidity and mortality (Koh *et al.,* 2012). Due to the foregoing, it is important to update on the susceptibility profile of bacteria recovered from wound infections from diabetic patients – with the overarching goal being to guide therapy, mitigate the emergence and community spread of antimicrobial resistance (AMR), as well as improve the prognosis of infected or affected patients in Abakaliki, Nigeria.

Materials and methods

Study population and pro-forma

Clinical samples of cases and symptoms of wound infections from both male and female volunteers within the age range of 18-80 years in the out-patient departments (OPD) of a tertiary hospital in Abakaliki, Nigeria was recruited for this study. The subjects were recruited upon approval by the Ethical Research Committee of the hospital under study. Oral informed consent was obtained from all participants using a structured pro-forma to obtain demographic data.

Collection and processing of specimens

A total of 32 diabetic males and 41 diabetic females were included in this study, and non-duplicate wound swab samples were collected randomly from the wound-inflicted patients including those suspected to be infected or who suffered diabetic clinical cases of wound infections. All samples were collected using sterile swab sticks and transported to the laboratory of Applied Microbiology Department of Ebonyi State University, Abakaliki, Nigeria for further microbiological investigations. Samples were cultured on selective culture media for the isolation and characterization of *Proteus specie, Escherichia coli, Pseudomonas aeruginosa, Salmonella* specie*, Shigella* species and *Staphylococcus aureus* – which are important human pathogens (Ponde*iet al.,* 2013; Ejikeugwu *et al*., 2022a).

Susceptibility studies

Antibiotic susceptibility studies was carried out according to the modified Kirby-Bauer disk diffusion technique using suspensions of the isolated test bacteria that was adjusted to 0.5 MacFarland turbidity standard. Aliquots of the test bacteria was swabbed on Mueller-Hinton (MH) agar plates using sterile swab sticks and single antibiotic susceptibility disks (Oxoid, UK) comprising: cefotaxime (30µg), amoxicillin-clavulanic acid (30µg), gentamicin (30µg), chloramphenicol (30µg), aztreonam (30µg), ceftazidime (30µg), imipenem (30µg), ofloxacin (30µg), erythromycin (30µg), Lincomycin (20µg), clindamycin (30µg), amikacin (30µg). All susceptibility test plates were incubated at 37°C for 18-24h and the zones of inhibition were measured and interpreted using the antibiotic breakpoint of the Clinical Laboratory Standard Institute (CLSI, 2017; Ejikeugwu *et al*., 2022b).

Determination of multiple antibiotic resistance index (MARI)

The formular: MARI=a/b was used to determine the multiple antibiotic resistance profile of the isolated resistant bacteria according to a previously used methodology (Ejikeugwu *et al*., 2022a). In the formular MARI, **"a"** represents the number of tested antibiotics that the resistant bacteria was resistant to, and **"b"** represents the total number of antibiotics for which the resistant bacteria was evaluated for.

Results

Table 1 and 2 shows the demographic distribution of the diabetic and non-diabetic participants included in this study, as well as the distribution of the isolated human bacterial pathogens recovered from the wound samples bacteriologically analyzed in this study. *Pseudomonas aeruginosa* was the highest isolated bacteria from the wound swab samples of the diabetic patients (38%) while the least isolated bacteria was *Shigella* species (3%). The highest number of isolated bacteria was from wound samples that emanated from diabetic patients (87%) while the non-diabetic patients had minimal bacteria contamination (13%).

The antibiotic resistance profile of the isolated *E. coli* and *P. aeruginosa* from the wound swab samples of both diabetic and non-diabetic patients is shown in Table 3 and 4. *E. coli* isolated from diabetic patients was 100% resistant to ofloxacin, ceftazidime, amoxycillin and azreonam, while that isolated from non-diabetic patients were resistant to ofloxacin (100%), ceftazidime (100%), chloramphenicol (100%) and amoxicillin (100%). On the other hand, the *P. aeruginosa* isolated from diabetic patients was resistant to ofloxacin (100%), ceftazimine (100%) and aztreonam (100%).

Table 5 and 6 shows the resistance profiles of the *Salmonella* species and *Proteus* species isolated from the wound samples of the participants in this study. The *Salmonella* species isolated

Table 1. Demography of diabetic and non-diabetic patients.

Demographic Parameter	Diabetic patients	Non-diabetic patients
Sex		
Male	32	11
Female	41	23
Total	**73**	**34**
Primary	14	19
Secondary	45	9
Tertiary	14	6
None	-	-
Total	**73**	**34**
Religion		
Christianity	73	34
Islamic	-	-
Traditional	-	-
None	-	-
Total	**73**	**34**
Occupation		
Trader	51	25
Farmer	14	4
Civil Servant	8	5
Total	**73**	**34**

Table 2. Frequency of human bacterial pathogens isolated from the wound samples.

Bacteria	No. Isolated n(%)	Diabetic n(%)
Escherichia coli	11 (11)	9 (82)
Pseudomonas aeruginosa	34 (34)	31 (82)
Salmonella specie	8 (8)	4 (50)
Proteus specie	20 (20)	17 (65)
Shigella specie	3 (3)	2 (66)
Staphylococcus aureus	24 (24)	24 (100)

Table 3. Resistance Pattern of *Escherichia coli* isolated from wound samples.

Antibiotic (µg)	Diabetic (n = 9) Resistance (%)	Non Diabetic (n = 2) Resistance (%)
Ofloxacin (30)	9 (100)	2 (100)
Ceftazidime (30)	9 (100)	2 (100)
Chloramphenicol (10)	4 (44)	2 (100)
Amoxicillin-clavulanic acid (10)	9 (100)	2 (100)
Gentamicin (30)	4 (50)	1 (50)
Aztreonam (5)	9 (100)	1 (50)
Cefotaxime (10)	4 (50)	1 (50)
Imipenem (10)	0 (0)	0 (0)

Table 4. Resistance Pattern of *Pseudomonas aeruginosa* isolated from wound samples.

Antibiotic (µg)	Diabetic (n = 31) Resistance (%)	Non Diabetic (n = 5) Resistance (%)
Ofloxacin (30)	31 (100)	5 (100)
Ceftazidime (30)	31 (100)	5 (100)
Chloramphenicol (10)	15 (44)	5 (100)
Amoxicillin-clavulanic acid (10)	31 (100)	5 (100)
Gentamicin (30)	15 (44)	4 (80)
Aztreonam (5)	31 (100)	5 (100)
Cefotaxime (10)	15 (44)	5 (100)
Imipenem (10)	0 (0)	0 (0)

Table 6. Resistance Pattern of *Proteus* species Isolated from wound samples.

Antibiotic (µg)	Diabetic (n = 17) Resistance (%)	Non Diabetic (n = 1) Resistance (%)
Ofloxacin (30)	17 (100)	1 (100)
Ceftazidime (30)	17 (100)	1 (100)
Chloramphenicol (10)	8 (44)	1 (100)
Amoxicillin-clavulanic acid (10)	17 (100)	1 (100)
Gentamicin (30)	10 (58)	0 (0)
Aztreonam (5)	17 (100)	1 (100)
Cefotaxime (10)	14 (82)	1 (100)
Imipenem (10)	0 (0)	0 (0)

from non-diabetic patients were resistant to ofloxacin (100%) and ceftazidime (100%) while *Salmonella* species recovered from the diabetic patients were resistant to ofloxacin (100%) and ceftazidime (100%). *Proteus* species isolated from both the diabetic and non-diabetic patients was resistant to ofloxacin (100%), ceftazidime (100%), chloramphenicol (100%), amoxicillin-clavulanic acid (100%) and aztreonam (100%).

The resistance profile of *Shigella* species and *S. aureus* isolated from both the diabetic and non-diabetic participants of this study is shown in Table 7 and 8. The *Shigella* species showed reduced susceptibility to ofloxacin (100%), aztreonam (100%), amoxicillin-clavulanic acid (100%) and ceftazidime (100%). For *S. aureus* isolates, reduced susceptibility was recorded against amikacin (100%), clindamycin (100%), chloramphenicol (83%), ofloxacin (75%) and lincomycin (70%). Table 9 shows the results of the multiple antibiotic resistance index (MARI) of the isolated bacterial pathogens in this study. On average, the MARI of the isolated bacteria was 0.7, indicating that the isolated bacteria showed multiple resistance to at least 7 out of the total antibiotics included in this study.

Discussion

Antimicrobial resistance (AMR) is one of the top 10 public

Table 7. Resistance Pattern of *Shigella* species isolated from wound samples.

Antibiotic (µg)	Diabetic (n = 2) Resistance (%)	Non Diabetic (n = 1) Resistance (%)
Ofloxacin (30)	2 (100)	1 (100)
Ceftazidime (30)	2 (100)	0 (0)
Chloramphenicol (10)	1 (50)	0 (0)
Amoxicillin-clavulanic acid (10)	2 (100)	1 (100)
Gentamicin (30)	1 (50)	1 (100)
Aztreonam (5)	2 (100)	0 (0)
Cefotaxime (10)	1 (50)	0 (0)
Imipenem (10)	0 (0)	0 (0)

Table 8. Resistance Pattern of *Staphylococcus aureus* isolated from wound samples.

Antibiotic (µg)	Diabetic (n = 24) Resistance (%)
Amikacin (30)	24 (100)
Clindamycin (30)	24 (100)
Lincomycin (30)	17 (70)
Chloramphenicol (30)	20 (83)
Vancomycin (30)	8 (33)
Erythromycin (15)	16 (66)
Ofloxacin (30)	18 (75)
Imipenem (10)	0 (0)

Table 9. Results of multiple antibiotic resistance index (MARI) of the isolated bacterial pathogens.

Bacteria	MARI
Escherichia coli	0.81
Pseudomonas aeruginosa	0.84
Salmonella species	0.77
Proteus species	0.69
Shigella species	0.54
Staphylococcus aureus	0.71

health threats of this century; and it also portends a threat to food security and the clinical efficacy of available antibiotics globally. Previous studies postulates that if nothing is done to mitigate it, AMR has the potential to plunge the world into a post-antibiotic era where no antibiotics will be clinically effective for treating and managing infectious diseases (Hansson and Brenthel, 2022). Some of the major fallouts of AMR include mortality, long hospitalization, increased cost of treatment and increased risk of acquisition and spread of nosocomial infections. The surveillance and detection of AMR is high and sustained in the clinical settings and the general environment of high-income countries (HICs) compared to the low- and middle-income countries (LMICs) where the tracking and reporting of AMR is still at a pitiable state. This current study investigated the frequency of AMR in some notable human pathogens recovered from the wound samples of both diabetic and non-diabetic patients in Abakaliki, Nigeria. The findings show that *P. aeruginosa* was the commonest isolates accounting for 38% of the total isolated pathogenic bacteria, and this was followed by *Proteus* spp. (26%), *S. aureus* (24%), *E. coli* (11%), *Salmonella* species (8%) and *Shigella* species (3%). The human pathogenic bacteria isolated from the wound samples investigated in this present study were akin to those recovered from surgical site infections – in which the organisms were found to be multiply-resistant to some commonly available antibiotics (Adegoke *et al*., 2010). From our study, *P. aeruginosa* was the most prevalent Gram-negative bacteria isolated from the wound samples. This is in agreement with the work of Christopher *et al*. (2022) who found that Gram negative bacteria were the most frequent microorganisms isolated from (diabetic) wound infections. The *E. coli, Salmonella* species, *Proteus* species and *P. aeruginosa* isolates recovered from the diabetic patients investigated in this study showed very high levels of resistance to some commonly available and clinically-relevant antibiotics including ofloxacin (100%), ceftazidime (100%), chloramphenicol (100%), amoxycillin (100%) and aztreonam (100%) which are used for the treatment of bacterial-related infections in the hospital. Similar levels of resistance of *E. coli, Salmonella* species, *Proteus* species and *P. aeruginosa* to the ofloxacin, amoxicillin, aztreonam, ceftazidime and chloramphenicol was also reported in the study conducted by Obeid *et al*., (2018) – which the isolated bacteria were found to be highly resistant to clinically-relevant antibiotics including those in the family: fluoroquinolones, beta-lactams, macrolides and aminoglycosides. On the other hand, we also reported a high resistance profile of the *Shigella* species and *S. aureus* isolates to some commonly available antibiotics. The *Shigella* species showed reduced susceptibility to ofloxacin (100%), aztreonam (100%), amoxicillin-clavulanic acid (100%) and ceftazidime (100%) while the *S. aureus* isolates showed high levels of reduced susceptibility to amikacin (100%), clindamycin (100%), chloramphenicol (83%), ofloxacin (75%) and lincomycin (70%). The high levels of resistance of *Shigella* species and *S. aureus* to clinically-relevant antibiotics corroborates the study conducted by Rahim *et al*. (2016) and Mehta *et al*. (2007) - in which Gram negative and Gram positive bacteria recovered from wound infections were found to be highly resistance to antibacterial agents including those in the classes: beta-lactams, aminoglycosides and fluoroquinolones. In a similar study, Bessa *et al*. (2015) reported that bacteria recovered from wound infections are unusually resistant to some clinically relevant antibiotics. On average, the isolated bacteria in this study showed multiple antibiotic resistance patterns to antibiotics in more than 6 different classes. This is also in line with the report by Adegoke *et al*. (2010) that bacteria isolated from wound infections particularly from surgical (wound) sites are becoming multiply resistant in nature.

Conclusion

This study has presumptively showed that wound infections are inundated by a diverse group of both Gram positive and

Gram negative bacteria – which are notoriously resistant to some clinically-relevant and available antibiotics. Since the pace at which bacteria develop resistance to antibiotics is not at par with the pace in the development of novel antibiotics, it is important to track the evolution and spread of AMR bacteria from wound infections in order to guide therapy, mitigate the evolution and spread of AMR and ensure good prognosis of the affected patients.

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