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Prevalence of Quinolone-susceptible Pseudomonas aeruginosa and Staphylococcus aureus in Delayed-healing DFU's in Ekpoma, Nigeria

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Issue:
Volume 22 - Issue 4 - April, 2010

Index:
WOUNDS. 2010;22(4):100–105.
Key words:
Quinolone
Pseudomonas aeruginosa
Staphylococcus aureus
Delayed-healing
DFU
Nigeria

Abstract: Aim. To investigate the prevalence and antibiogram of Pseudomonas aeruginosa and Staphylococcus aureus from delayed-healing foot ulcers among patients with diabetes in Ekpoma. Methods. Using standard aseptic microbiological methods, 220 delayed-healing diabetic foot ulcer samples were analyzed for bacteria isolation, identification, and susceptibility before and 12 weeks after antibiotic administration. Chi-squared ($\alpha = 0.01$) was used to test for statistical significance. Results. Out of the 220 samples analyzed, 181 (82.3%) were infected (P aeruginosa [41.8%]; S aureus [30%]; co-infection of P aeruginosa and S aureus [10.5%]). Wound healing was significantly ($P < 0.01$) dependent on the presence of P aeruginosa and S aureus in the study population. S aureus and P aeruginosa showed the highest (74.2% and 71.3%, respectively) and lowest (38.2% and 34.8%) susceptibilities to levofloxacin and sparfloxacin, respectively. P aeruginosa was 68.7% susceptible to rifampicin; 53% to erythromycin, 52.2% to

vancomycin; 38.3% to ceftriazone; 36.5% to cefuroxin; and 32.2% to oxacillin. S aureus was 51.7% susceptible to rifampicin, 37.1% to cefuroxin; 33.7% to ceftriazone; 28.1% to vancomycin; and 25.8% to oxacillin. Twelve weeks after antibiotic administration, 54% of samples had no growth and showed accelerated wound healing; 26.7% yielded P aeruginosa, while 19.3% yielded S aureus. Conclusion. Delayed-healing diabetic foot ulcers in Ekpoma are colonized by levofloxacin- and sparfloxacin-susceptible P aeruginosa and S aureus. Microbial load reduction due to appropriate antibiotic administration contributed to the acceleration of the wound healing process for 54% of patients who participated in the follow-up procedures. Surveillance with improved diagnostic facilities is recommended.
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Wound or tissue injury is one of the most common causes of hospitalization in non-diabetic patients and patients with diabetes living in sub-Saharan Africa.[1,2] A diabetic foot infection (DFI) is a common cause for hospital admission among patients with diabetes in Ekpoma, Edo State, Nigeria.[1,3] Foot infection is rapidly becoming one of the most common complications among patients with diabetes and approximately 40% to 72% of all lower extremity amputations are related to DFI.[4] A number of studies on the bacteriology of DFIs have found that S aureus and the beta-hemolytic streptococci (groups A, B, C, and G) are predominant, and in some cases the sole bacterial pathogens associated with wound infections in patients with diabetes.[5,6] P aeruginosa is prominent among complex colonizing flora of chronic wounds.[7,8] The exact importance of microorganisms in nonhealing wounds that do not show clinical signs of infection is presently being questioned with respect to whether the density of microorganisms, the presence of specific pathogens, or total absence of microorganisms could be the critical factor determining whether a wound is likely to heal or experience delayed healing.[9] Although appropriate systemic antibiotics are considered essential for the treatment of nonhealing clinically infected wounds, there is a debate regarding the relevance and use of systemic and topical antibiotics, or the use of topical antiseptics in the treatment of nonhealing, noninfected wounds.[9] Onuminya and Onuminya[1] attributed treatment failures in wound management to recurrent infection due to persisting local ischemia and foreign body reaction (gauze or sequestrum). Inadequate debridement of the infected tissues, immunocompromised patient status, and emergence of multidrug resistant bacterial pathogens[10] due to indiscriminate abuse of available inexpensive antibiotics might play a crucial role in recurrent infections[11] in Ekpoma. Now that methicillin-resistant S aureus (MRSA) has emerged as a common cause of hospital and community acquired foot infections,[2] it is therefore necessary to include anti-staphylococcal agents when selecting antibiotics for presumptive treatment of DFIs especially in developing countries with inadequate diagnostic facilities. A need exists for effective local antibiotic resistance surveillance in order for healthcare providers to improve the management of these infections. While data on the etiology of nondiabetic wound infection in Ekpoma are scarce,[1,3] the authors are not aware of any similar report on diabetic foot infections in Ekpoma. The following study investigated the predominance of P aeruginosa and S aureus in DFI patients and determined the susceptibility profile of these two bacteria to commonly used antibiotics. The goal of the study sought to improve the management and care of patients with diabetes with infected, delayed-healing foot ulcers in Ekpoma.

Methods

Ethical considerations. The ethics committee of Searchlight Medical Diagnostic Center (SMDC) in Ekpoma, Edo State, Nigeria approved the study. Sampling area. Ekpoma is a University town situated 90 km north of Benin, the capital city of Edo State, Nigeria. It has few private clinics and does not have specialists or a referral hospital. All referral cases are usually sent to Irrua Teaching Hospital located in a nearby community. In the present study, wound healing was considered delayed when the normal wound healing process of homeostasis, inflammation, granulation, and remodeling lasted more than 12 weeks from the day the tissue injury was first sustained.[12] Sample collection and inclusion criteria. Two hundred-twenty (220) delayed-healing diabetic foot wound swab samples were aseptically collected and analyzed at SMDC, Ekpoma. The swab sampling technique has limitations because it collects superficial contaminants that do not reflect the deeper, infecting microorganisms. However, since synergy undoubtedly occurs in polymicrobial wounds, superficial microorganisms might contribute to wound pathogenesis. Subjects who qualified for inclusion were adult patients with diabetes who had delayed-healing DFIs. Healthcare providers in Ekpoma and its vicinity referred the patients to SMDC to undergo isolation, identification, and susceptibility testing to determine the microbial etiology of the delayed-healing DFIs. Ekpoma residents with diabetes and a delayed-healing DFI who presented to SMDC with similar health conditions without the healthcare provider referral were also included. Patients with foot ulcers who did not have diabetes were excluded from this study. Sample analysis. An aseptic and standard microbiological method contained in our previous report[13] was adopted in media preparation and sample inoculation. MacConkey agar, blood agar base, nutrient agar, and Mueller-Hinton sensitivity testing agar medium were prepared according to manufacturer's (OXOID Limited UK) instructions. They were sterilized at 121˚C for 15 minutes holding time in an autoclave. Sheep blood agar (10%) was prepared by mixing 10-mL fresh sheep blood with 90-mL molten blood agar base at ~45˚C. Approximately 20 mL of each medium was dispensed on a sterile disposable plastic Petri dish and allowed to set. Samples were inoculated unto MacConkey, nutrient, and blood agar plates, respectively, for bacterial isolation. They were all incubated at 37 ± 3˚C for 18–24 hours. Direct gram smears were made on a microscope slide and wet mounts were prepared and examined microscopically. Significant mixed growth of bacteria colonies (more than 25 colonies per plate) were separated into single colonies by obtaining purity plates. All suspect colonies for P aeruginosa and S aureus were identified according to Cowan and Steel's Manual for the Identification of Medical Bacteria, as revised by Barrow and Feltham.[14] Antibiotic susceptibility test. Commercially prepared antibiotic disc (Difco) was used. Kirby-Bauer's National Committee for Clinical Laboratory Standards[15] modified disc diffusion technique for the antibiotic susceptibility test was adopted in this investigation. Control of test performance outlined by NCCLS[16] was strictly followed. After incubation at 35˚C for 18–24 hours, zone sizes were measured and interpreted using NCCLS standards.[16] The criterion for antibiotic inclusion was based on first line broad- and narrow-spectrum antibiotics commonly used for soft tissue infection in Ekpoma,[11] which was in line with the guideline for antibiotic selection during susceptibility testing by the National Committee for Clinical Laboratory Standards.[15,16] The antibiotics included and each disc's antibiotic contents were: ciprofloxacin (5 µg), ofloxacin (5 µg), levofloxacin (5 µg), moxifloxacin (5 µg), vancomycin (30 µg), sparfloxacin (5 µg), gentamicin (10 µg), ampicillin-cloxacillin (10 µg), cotrimoxazole (300 µg), oxacillin (1 µg), ceftriaxone (30 µg), cefuroxime (30 µg), rifampicin (5 µg), and erythromycin (15 µg). The chi-

square test was used to test for statistical significance. Sample collection, analysis, and susceptibility testing protocols described above were repeated 12 weeks later when each patient returned to SMDC for follow-up laboratory investigation.

Results

Overall, an 82.3% prevalence (n = 181) of microbial infection in diabetic foot ulcers in Ekpoma was found before antibiotic administration, and 46% (n = 101) prevalence was found after antibiotic administration over the 12-week study period. Of the 220 samples that were analyzed before antibiotic administration, 92 (41.8%) yielded pure and heavy growth of P aeruginosa, 66 (30%) yielded moderate growth of S aureus, and 23 (10.5%) yielded significant growth of both P aeruginosa and S aureus. Twelve weeks after antibiotic administration, 70 patients did not return for follow up. Of the 150 patients who reported for follow up, 40 (26.7%) yielded pure growth of P aeruginosa and 29 (19.3%) yielded pure growth of S aureus. Eighty-one (54%) samples yielded no growth after a 48-hour incubation and demonstrated accelerated healing. P aeruginosa and S aureus demonstrated similar susceptibility patterns (Table 1). Other unidentified bacteria isolates showed insignificant growth after the 48-hour incubation. The results depict a trend of decreasing susceptibility of S aureus and P aeruginosa against the following quinolones used: levofloxacin > moxifloxacin > ofloxacin > ciprofloxacin > sparfloxacin (Table 1). Again, P aeruginosa was less susceptible (36.5%) to cefuroxin than S aureus (37.1%), whereas P aeruginosa was more susceptible (38.3%) to ceftriazone than S aureus (33.7%). Both P aeruginosa and S aureus were poorly susceptible to gentamycin (8.7% and 9%, respectively). The complete list of P aeruginosa and S aureus percentage susceptibility ratios are shown in Table 1. Susceptibility ratios of S aureus from the authors' previous study in Ekpoma11 compared to the present investigation are shown in Table 2. The association between P aeruginosa and S aureus in the study population was statistically significant ($P < 0.01$).

Discussion

Exposed, devitalized (eg, ischemic, hypoxic, or necrotic), and immunocompromised subcutaneous tissue provides a favorable condition for growth of environmental and surrounding skin microflora.[17] Plantar ulcers associated with diabetes are susceptible to infection due to high incidence of mixed wound microflora.[18] The observed 82.3% prevalence of infected, delayed-healing diabetic foot ulcers in this study is slightly higher than 72% reported in India[4] and 12% report from western countries.[19] This observed difference in DFI may be ascribed to poor socioeconomic conditions, inadequate facilities for diabetes care and education and also due to walking barefoot. Ekpoma lacks good diabetic wound clinics to control the multitude of microbial and host factors (ie, type, location, size, wound depth, the extent of nonviable exogenous contamination, level of perfusion to the wound, patient's general health and immune status, microbial load, and the combined level of virulence expressed by the types of microorganisms involved), which may have minimized the progression of wounds to infected state. The involvement of foreign materials such as sutures, dirt, grafts, or prosthetic devices may increase the risk of infection in clean wounds.[20] In this study, the 41.8% prevalence of P aeruginosa is slightly higher than earlier African and Indian reports of 33% and 36%.[21,22] However, it is different from the 73.1% found in Iran.[23] These differences may be due to the

decrease in the level of general hygienic measures in addition to the mass production of low-quality antiseptic and medicinal solutions for wound treatment. Sub-inhibitory antibiotic concentration in wounds may also provide optimal conditions for the selection and persistence of multidrug resistant P aeruginosa strains. Meanwhile, the observed 30% prevalence of S aureus is higher than the 10.3% reported in Iran,[22] lower than 60% from the United States,[2] and lower than two reports (48% and 70%) from nondiabetic wounds in Ekpoma.[1,24] However, there was a 10.5% prevalence of P aeruginosa and S aureus co-infection. This is similar to the authors' previous report of 11% co-infection of S aureus with other bacteria isolated from female genital wounds in Ekpoma.[11] The problem of polymicrobial infections has been reported extensively, especially in Ekpoma, and multidisciplinary surveillance with improved diagnostic facilities were recommended as panacea for proper management, prevention, and control of reported diseases.[25–27] Conditions for co-infection of P aeruginosa and S aureus, as Lipsky et al[2] have reported, are long duration, nonhealing wounds that undergo prolonged periods of broad-spectrum antibiotic therapy, and wounds that become macerated. Microbial synergy in mixed infected wounds known to increase the net pathogenic effect and hence the severity of the wound may have contributed in the establishment of delayed-healing wound ulcers among the studied population. The emergence of multidrug-resistant P aeruginosa in delayed-healing diabetic foot infections in Ekpoma has been observed (Table 1). The multi-drug resistance of P aeruginosa is a source of serious concern because they are resistant to antibiotics available in Ekpoma. This observation might be explained by indiscriminate use of antibiotics (self medication) most likely due to lack of prophylactic antibiotics policy in Ekpoma. Expensive medical services by private clinics, lack of facilities for laboratory confirmation of etiology and corresponding susceptibility testing may also outline the emergence of multidrug resistant P aeruginosa in delayed healing diabetic foot infections in Ekpoma. P aeruginosa may also be developing resistance to commonly used antibiotics faster than the rate at which new and improved antibiotics are made available to healthcare providers in Ekpoma. Careful use of appropriate broad-spectrum antimicrobial agents may lead to successful treatment of clinically infected chronic wounds characterized by polymicrobial flora. This may explain why 70 (31.8%) of the studied population never returned for follow up, and of the 150 patients who returned after 12 weeks, 81 (54%) whose samples did not show growth experienced accelerated wound healing. It is likely that further promising results might have been seen if the remaining 46% whose wounds showed growth after 12 weeks had been followed up. The 62.5% susceptibility of P aeruginosa to ciprofloxacin observed in this study is lower than the 70%–80% reported from a similar US study.[28] It is slightly different from 79% reported in Bangladesh,[29] but similar to 61.5% reported in France.30 The observed 71.3% susceptibility of P aeruginosa to levofloxacin may indicate less abuse by Ekpoma inhabitants. The decreasing susceptibility range (71.3%–34.8%; Table 1) of P aeruginosa against quinolones observed in this investigation may be a useful guide in antibiotic selection in the treatment of diabetic foot and other nondiabetic and delayed-healing wound infections involving P aeruginosa. The observed 8.7% susceptibility of P aeruginosa to gentamycin in this study is lower than the reports of 25% and 78.4% susceptibility in Russia as well as Trinidad and Tobago, respectively.[31,32] P aeruginosa was 100% resistant to cotrimoxazole. This may show the level of abuse of cotrimoxazole. The observed 74.2% susceptibility of Levofloxacin may show that the drug is new in Ekpoma. In some mixed wound infections where excessive populations of gram-negative bacteria are likely to be present, careful selection of antibiotics is required since some are known to influence more endotoxin liberation (cell wall agents) than do inhibitors of protein synthesis.[20] The use of broad-spectrum

topical antibiotics to treat wounds that are failing to heal or wounds that are at risk of infection is justified on the basis that they provide a high concentration at the local site; they avoid systemic allergic reactions and they also avoid more-widespread effects on endogenous bacteria.[33]

Comparative analysis of the antibiotic susceptibility of S aureus previously isolated from genital wound[11] and S aureus presently recovered from diabetic foot infections showed a remarkable decrease in susceptibility (Table 2). These decreases in susceptibility may imply that previous recommendations[11] may not have been fully embraced in treatment of wound infected with S aureus and the fact that laboratory results may not always translate to therapeutic success.[34]

Conclusion

The delayed-healing diabetic foot ulcers (82.3%) that were analyzed in Ekpoma were infected with P aeruginosa (41.8%), S aureus (30%), and both P aeruginosa and S aureus (10.5%). Administration of appropriate antibiotics accelerated the healing process of 81 (54%) of 150 patients who returned for follow up at 12 weeks. The antibiotic susceptibility profiles of isolated P aeruginosa and S aureus were low due to indiscriminate use of antibiotics, expensive medical services, and lack of laboratory facilities. Delayed-healing diabetic foot ulcers in Ekpoma were colonized by levofloxacin- and sparfloxacin-susceptible P aeruginosa and S aureus.

Acknowledgements

We are grateful to management and staff of Searchlight Medical Diagnostic Center for approving this study and providing the resources and enabling environment for this research. We also thank all of the patients whose consent made this investigation a reality. Finally, we are heavily indebted to all clinics in Ekpoma whose patients participated in this investigation.