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Research Article

Identification and determination of antibiotic resistance of pathogenic bacteria Isolated from Septic Wounds

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Abstract: Wound infection is a global cause of morbidity and mortality across all wound types. Therefore, efficient diagnosis and treatment of wound infection are essential. This study was carried out to identify the pathogenic bacteria in infected wounds of the patient's attending Sebha city hospitals (Libya) and to determine their resistance profile to the most common antibiotics used in therapy. A total of sixty wound swab specimens were collected and cultured, of which 39 samples showed bacterial growth. Three different species of bacteria were isolated. *Staphylococcus aureus* 21 (53.9%) were the most common organisms followed by *Pseudomonas aeruginosa* 10 (25.6%) *and Staphylococcus epidermidis* 8 (20.5%). The antibiotic susceptibility test of the bacterial isolate was performed by Kirby-Bauer disk diffusion method. Results showed that 90.5% of the *Staphylococcus aureus* isolates were resistant to vancomycin, 61.9% to tetracycline, 57.1% to amoxicillin, 52.4 % to methicillin, 42.9 to erythromycin and 23.8% to streptomycin. 87.5% of the *Staphylococcus epidermidis* isolates were resistant to vancomycin, 75% to methicillin, 62.5% to tetracycline, 50% to streptomycin 37.5% to amoxicillin, and erythromycin. All the *Pseudomonas aeruginosa* isolates were sensitive to ciprofloxacin and highly resistant 90-100% to other antibiotics tested Amoxicillin, Nalidixic acid, Streptomycin, and Tetracycline. The high rate of multiple antibiotic resistance was observed in all bacterial species recovered.

Keywords: Septic wounds, Pathogenic Bacteria, Identification and Determination of Antibiotic Resistance.

1. Introduction

Human skin has inherent properties that are important in preventing infection and promoting healing in wounds. The structure and function of the skin are not uniform, and specific adaptations are found at different anatomical sites. Human skin is a multifunctional organ that provides sensation, thermoregulation, biochemical, metabolic, immune functions, and physical protection (Wysocki, 2002). Intact skin is the perfect defense against bacterial invasion, but damage to the skin allows bacteria, fungi, and yeasts to enter. More than 200 different species of bacteria normally live on the skin (Benbow, 2010). When one or more microorganisms multiply in the wound, local and systemic responses occur in the host, which can lead to infection and a subsequent delay in healing (Angel *et al*., 2011). Maintaining the bacteria at a level at which the host is in control is an important part of avoiding wound infection (Cutting, 2010) and an open wound provides a moist, warm and nutritious environment perfect for microbial colonization and proliferation (Edwards & Harding, 2004). Infection of a wound may be defined as invasion of organisms through tissues following a breakdown of local and systemic host defenses. Major wound infection is seen when a wound discharges pus and may need a secondary procedure to be sure of adequate drainage; there may be systemic signs or delay in return home. In minor wound infection, there is a discharge of pus or serous fluid without associated excessive discomfort or systemic signs. Wound infections are the commonest and most troublesome disorder of wound healing (Ahmed *et al*., 2007). Repeated wound infections can lead to depression and anxiety for the patient due to increased systemic symptoms and an obviously visible deterioration of the wound (EWMA, 2008). They are expensive and cannot heal, increasing treatment costs

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and the demand on nursing resources (Bowler *et al*., 2001). Therefore, efficient diagnosis and treatment of wound infection are essential. However, this can prove to be challenging and as there is no expert consensus on the best assessment methods, it is entirely dependent upon the skill of the individual clinician (Fierheller & Sibbald, 2010). We live in a predominately microbial world with the human body containing an estimated 1014 microbial cells. Although these microbiotas have an important role to play in the maintenance of health, they nonetheless have the potential to cause disease given the opportunity. The majority of cutaneous wounds are colonized (some heavily) with both aerobic and anaerobic indigenous microorganisms that are found colonizing the mucosal surfaces, such as the gut and oral cavity. The number of microbial species identified in cutaneous and soft tissue infections continues to increase (Dennis *et al*., 2005). Since, the late nineteenth century, it has been recognized that the principal pathogens associated with wound infections are *Staphylococcus aureus*, *Streptococcus* species, anaerobes and *Pseudomonas aeruginosa* (EWMA, 2005). Wound infection is a global cause of morbidity and mortality across all wound types and data related to the associated prevalence/incidence of wound infection, therefore, demands our attention (AAWC, 2008). There is clearly a need for further development of the criteria for early recognition of wound infection. Access to more precise and sophisticated assessment tools will increase the possibility for prompt diagnosis and assist with the obvious benefit of reducing patient morbidity (EWMA, 2005). Patients who present to the hospital with severe infection or whose infection is progressing despite empirical antibiotic therapy should be treated more aggressively, and the treatment strategy should be based upon the results of appropriate Gram stain, culture, and drug susceptibility analysis. In the case of *S. aureus*, the clinician should assume that the organism is resistant, because of the high prevalence of community-associated MRSA strains, and agents effective against MRSA (i.e., vancomycin, linezolid, or daptomycin) should be used (A-I). Step down to treatment with other agents, such as tetracycline or trimethoprim-sulfamethoxazole, for MRSA infection may be possible, based on results of susceptibility tests and after an initial clinical response (Dennis *et al*., 2005). Non-healing wounds are a significant problem for health-care systems worldwide (Posnett *et al*., 2009).

Infection is one of the most frequent complications of non-healing wounds. It can jeopardise the progression towards healing, result in longer treatment times and increase resource use. In the worst cases, it can result in a major amputation or a life-threatening condition. Wounds are disposed to infection, as the exposure of subcutaneous tissue following a loss of skin integrity provides a moist, warm, and nutrient-rich environment, which is conducive to microbial colonisation and proliferation. Consequently, use of antimicrobial agents is important in wound management. Inappropriate use of antimicrobials (especially antibiotics) creates an environment for the selection of resistance against the currently available antimicrobial products, with the potential consequence of significantly jeopardising patients' health status. The development of so-called 'superbugs' is foreseeable and is the background for increased political involvement[5–7] (Gottrup *et al*., 2013).

The control of wound infections has become more challenging due to widespread bacterial resistance to antibiotics and to a greater incidence of infections caused by methicillin-resistant *Staphylococcus aureus*, polymicrobic flora and by fungi. The knowledge of the causative agents of wound infection has therefore proved to be helpful in the selection of empiric antimicrobial therapy and on infection control measures in health institutions (Shittu *et al*., 2002). The aim of this study was to identify the bacterial pathogens in infected wounds in the patient's attending Sebha city hospitals (Libya) and to determine their resistance profile to the most common antibiotics used in therapy.

2. Materials and methods

2.1 Specimen collection

The wound samples were collected by using sterile cotton swabs. A total of 60 sterile cotton swab samples of different types of infected wounds were collected from patients (males and females of different ages) who attended Sebha Hospital (15 samples) and two other primary care health centers in the Sebha city, Althanwia center (21 samples) and Almenshia center (24 samples). All microbiological procedures were conducted in the Sebha Central Medical Laboratory at (Microbiology department). All samples were inoculated on 5% blood agar as well as on Nutrient agar and MacConkey agar plates and examined by Gram smear staining. Cultures were aerobically incubated at 37^0C overnight. Positive cultures were identified using standard diagnostic microbiological and biochemical laboratory test methods like Catalase, Coagulase, DNase, Oxidase tests according to standard procedures described by Cowan & Steels (Barrows & Feltham, 2003) and (Cheesbrough, 2000).

2.2 Antibiotic sensitivity testing

This was performed using the standard disk diffusion method (Kirby-Bauer method) as described in the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, 2004). All species isolated were tested for antibiotic sensitivity against commonly used antibiotics using commercial discs (Oxoid Limited, England) containing: oxacillin (OX), amoxicillin (AML), erythromycin (E), ciprofloxacin

(CIP), vancomycin (VA), chloramphenicol (C), tetracycline (TE), nalidixic acid (NA), streptomycin (S). The organisms under investigation were cultured in Mueller-Hinton sensitivity testing agar, then 5-7 different antibiotic discs were placed on the media about two centimeters apart. After overnight incubation at 37^0C aerobically the culture was examined for zone of inhibition of bacterial growth around the respective disks which was measured in millimeters. Using the interpretative chart, interpret the zone sizes of each antibiotic reporting the organisms as resistant, intermediate, sensitive.

3. Results

A total of 39 samples were positive for the culture out of 60 cotton swab samples collected from patients and 39 bacteria were isolated, they were Gram-positive cocci 29 (74.4%) and Gram-negative bacilli 10 (25.6%). Most frequently isolated organism was *Staphylococcus aureus* 21 (53.9%), followed by *Pseudomonas aeruginosa* 10 (25.6%) *and Staphylococcus epidermidis* 8 (20.5%) (Table 1). The antibiotic susceptibility test of the bacterial isolate was performed by Kirby-Bauer disk diffusion method. Results showed that 90.5% of the *Staphylococcus aureus* isolates were resistant to vancomycin, 61.9% to tetracycline, 57.1% to amoxicillin, 52.4% to oxacillin/methicillin, 42.9 to erythromycin and 23.8% to streptomycin. 87.5% of the *Staphylococcus epidermidis* isolates were resistant to vancomycin, 75% to oxacillin/ methicillin, 62.5% to tetracycline, and 50% to streptomycin 37.5% to amoxicillin, and erythromycin (Table 2). All the *Pseudomonas aeruginosa* isolates were sensitive to ciprofloxacin and their resistance to other antibiotics tested (Amoxicillin, Nalidixic acid, Streptomycin, Tetracycline, was between 90-100% (Table 3).

Table 1. Species of bacteria isolated (number and percentage).

Bacterial species	No	Percentage (%)
Staphylococcus aureus	21	53.9%
Staphylococcus epidermidis	8	20.5%
Pseudomonas aeruginosa	10	25.6%
Total number	39	100%

Table 2. Results of antibiotic sensitivity testing of gram-positive isolates.

Species/ Antibiotic	OX		AML		VA		S		TE		E	
	S	R	S	R	S	R	S	R	S	R	S	R
	%		%		%		%		%		%	
S. aureus	47.6	52.4	42.9	57.1	9.5	90.5	76.2	23.8	38.1	61.9	57.1	42.9
S. epidermis	25	75	62.5	37.5	12.5	87.5	50	50	37.5	62.5	62.5	37.5
Total	100		100		100		100		100		100	

OX = Oxacillin; **TE =** Tetracycline; **S =** Streptomycin; **AML =** Amoxicillin; **E =** Erythromycin; **VA =** Vancomycin; **S =** Sensitive, **R =** Resistant

Table 3. Results of antibiotic sensitivity testing of gram-negative isolates.

Species/ Antibiotic	AML		NA		S		TE		CIP	
	S	R	S	R	S	R	S	R	S	R
	%		%		%		%		%	
P. aeruginosa	10	90	10	90	10	90	10	90	100	0
Total	100		100		100		100		100	

AML = Amoxicillin; **NA** = Nalidixic acid; **S =** Streptomycin; **TE** = Tetracycline; **CIP** = Ciprofloxacin; **S** = Sensitive; **R =** Resistant

4. Discussion

Wound microbiology may be considered a complex and sometimes misunderstood area in clinical medicine, not least because a wound provides an environment in which the microbial ecosystem is very dynamic and unstable (AAWC, 2008). Wound infections can be caused by different groups of microorganisms like bacteria, fungi, and protozoa. However, different microorganisms can exist in polymicrobial communities, especially in the margins of wounds and chronic wounds. The infecting microorganism may belong to aerobic as well as anaerobic group (Zafar *et al*., 2008). Wound infection plays an important role in the development of chronicity, delaying wound and healing (Bessa *et al*., 2013).

In this study gram-positive pathogens isolated from wound infections, staphylococci found to be a major causative agent with a ratio of 74.4% followed by *Pseudomonas aeruginosa* 25.6%. This is in agreement with the results obtained by Ozkuyumcu *et al*., (1989). The *S. aureus* is the leading etiologic agent of wound infection (53.9%) as reported by other workers (Shittu *et al*., 2002). The high prevalence of *S. aureus* infection may be because it is an endogenous source of infection. Infection with this organism may also be due to contamination from the environment (Mama *et al*., 2014). The control and management of infection is a complex and important aspect of wound care. Although antibiotics have been of great value in the treatment and in prophylaxis to prevent infections, the timing of administration, choice of antimicrobial agents, duration of administration have clearly defined the value of antibiotics in reducing wound infections (Nichols, 2001). Even though treatment, especially in life-threatening situations is usually empiric employing broad-spectrum antibiotics, increasing rates of antibiotic resistance among pathogens have been a major impediment to the success of empiric treatment, resulting in treatment failure hence increasing treatment cost (Thanni *et al*., 2003). The widespread uses of antibiotics, together with the length of time over which they have been available have led to major problems of resistant organisms contributing to morbidity and mortality. Knowledge of the causative agents of wound

infection in a specific geographic region will, therefore, be useful in the selection of antimicrobials for empiric therapy (Manikandan and Amsath, 2013). In our study, the antibiotic susceptibility tests displayed that the isolated bacteria were highly resistant to common antibiotics. Results showed that 90.5% of the *Staphylococcus aureus* isolates were resistant to vancomycin, 61.9% to tetracycline, 57.1% to amoxicillin, 52.4% to methicillin, 42.9% to erythromycin and 23.8% to streptomycin. Also, 87.5% of the *Staphylococcus epidermidis* isolates were resistant to vancomycin, 75% to methicillin, 62.5% to tetracycline, 50% to streptomycin, 37.5% to amoxicillin, and erythromycin. Considerable variations in the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) exist between institutions and between geographic areas. In Ethiopia, Godebo *et al*., (2013) found that about (76.7%) of *S. aureus* was Oxacillin/Methicillin-resistant while (16.4%) were Vancomycin-resistant. In some areas of Europe high proportions (60-70%) of CNS were methicillin resistant (Stefani and Varaldo, 2003). Oxacillin has been one of the main drugs used for the treatment of staphylococcal infections; however, a large number of *S. aureus* and CNS isolates of nosocomial origin are resistant to this drug. Methicillin resistance is encoded by the mecA gene, which is inserted in the SCCmec cassette (Martins and Cunha Mde, 2007). Methicillin-resistant staphylococci are mostly resistant not only to all beta-lactams but also to a wide range of other antibiotics and have emerged as major nosocomial pathogens during the past two decades (Stefani and Varaldo, 2003). In this study, all the *Pseudomonas aeruginosa* isolates were sensitive to ciprofloxacin and this is in agreement with the results obtained by Ndip *et al*., (2005) who found that the susceptibility of *Pseudomonas aeruginosa* isolates to ciprofloxacin (98%), 90 to 100% of our *Pseudomonas aeruginosa* isolates were resistant to the four other antibiotics tested (Amoxicillin, Nalidixic acid, Streptomycin, Tetracycline). Which is much higher compared to the results obtained by (Ndip *et al*., 2005). Such high antimicrobial resistance is probably due to the widespread empiric use of these broad-spectrum antibiotics in the study area among patients. These drugs are relatively cheap and are sold by unauthorized persons and without a prescription. Their high level of misuse accounts for the high levels of resistance observed. Antibiotic-resistant bacteria can emerge in three main ways--by the acquisition of new genes *via* transposons or horizontal gene transfer, by selection of resistant variants and by selection of naturally resistant strains. In order to minimize the emergence of antibiotic resistance during therapy, it is important to try and avoid antibiotics, which encourage the transfer of resistance genes, to avoid selection of resistant variants from susceptible pathogens and to avoid ablation of antibiotic-susceptible normal flora. However, implementing these objectives is not always easy (Williams and Sefton, 1999).

5. Conclusion

In this study gram-positive pathogens isolated from wound infections, Staphylococci found to be a major causative agent with the ratio of 74.4% followed by *Pseudomonas aeruginosa* 25.6%. The isolated bacteria were highly and multiple drugs resistant and it were difficult to treat. Knowing the distribution and the drug resistance pattern of the pathogen is of paramount importance in guiding the clinical treatment.

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