LITERATURE REVIEW

Introduction  
Wound care constitutes an important part of routine care given by health professionals to the community population (Meaume S, Keriheul JC, Fromantin I, Teot L. Workload and prevalence of open wounds in the community: French Vulnus initiative. **J Wound Care** 2012;21:62–6.**)**. An effective management of wounds, especially chronic wounds, in the health care setting can have an impact in the population health, reducing morbidity and improving function and quality of life. Wounds presented by patients vary from one setting to another, ranging from acute surgical wounds, traumatic wounds such as those that occur following an accident, burn wounds or chronic wounds such as diabetic foot, leg and  
pressure ulcers. All wounds are contaminated with microorganisms that are part of the saprophytic microflora of the skin and the type and quantity of these microorganisms vary from one wound to another (Cooper R, Lawrence JC. The isolation and identification of bacteria  
from wounds. **J Wound Care** 1996;5:335–40.**)**. Some important factors such as origin, body location, size and duration of the wound should  
be taken into account in the wound management because of their impact on wound colonisation and infection (White RJ, Cooper R, Kingsley A. Wound colonization and infection: the role of topical antimicrobials. **Br J Nurs** 2001;10:563–78.**)**. Microbial colonisation of wounds is characterised by the presence of multiplying microorganisms on the surface of a wound, but with no immune response from the host (Edwards R, Harding KG. Bacteria and wound healing. **Curr Opin****Infect Dis** 2004;17:91–6., Bowler PG, Duerden BI, Armstrong DG. Wound microbiology and associated approaches to wound management. **Clin Microbiol Rev**2001; 14:244–69) and with no associated clinical signs and symptoms. Differently, wound infection depends on the pathogenicity and virulence of the microorganisms and on the immune competency of the host and it is determined by the presence of clinical signs of infection such as erythema, pain, tenderness, heat, oedema,  
cellulites and abscess/pus (European Wound Management Association (EWMA). Position document: identifying criteria for wound infection. London: MEP Ltd, 2005., Collier M. Recognition and management of wound infections. 2004.  
URL http://www.worldwidewounds.com/2004/january/Collier/  
Management-of-Wound-infections.html [accessed on 12 October  
2012]). Therefore, wound infection results in active disease that is likely to delay the wound healing process (Beldon P. Recognising wound infection. Nurs Times 2001;97:3–4.). Moreover, despite these common criteria to identify wound infection, clinicians should be aware that  
each wound type may present different clinical signs of infection. Thus, the presence of microorganisms per se is not indicative of wound infection (Howell-Jones RS, Wilson MJ, Hill KE, Howard AJ, Price PE, Thomas DW. A review of the microbiology, antibiotic usage and  
resistance in chronic skin wounds. J Antimicrob Chemother 2005;  
55:143–9.). However, the probability that a critical microbial load may directly contribute to the non healing outcome in both acute and chronic wounds has been considered and evidence has been shown (Bowler PG, Duerden BI, Armstrong DG. Wound microbiology and associated approaches to wound management. Clin Microbiol Rev2001;14:244–69., Serralta VW, Harrison-Balestra C, Cazzaniga AL, Davis SC, Mertz PM. Lifestyles of bacteria in wounds: presence of biofilms? Wounds2001;13:29–34.). Other studies in other polymicrobial chronic infections suggest that the presence of specific pathogens is more important that the bacterial burden (Davies CE, Hill KE, Newcombe RG, Stephens P, Wilson MJ, Harding KG, Thomas DW. A prospective study of the microbiology of chronic venous leg ulcers to reevaluate the clinical predictive valueof tissue biopsies and swabs. Wound Repair Regen 2007;15:17–22., Dowd SE, Wolcott RD, Sun Y, McKeehan T, Smith E, Rhoads D. Polymicrobial nature of chronic diabetic foot ulcer biofilm infections determined using bacterial tag encoded FLX amplicon pyrosequencing (bTEFAP). Generally, after the clinical diagnosis of infection is made, culture is recommended to identify the causative organisms and guide antibiotic therapy.

In this study, we investigated the bacterial profile and assessed their antimicrobial susceptibility pattern of infected wounds presented by patients from who swab samples were collected and analysed by Microbiology laboratory of Pescara Hospital, in central Italy, during a 6-month period. The aim was to identify the bacterial species present within the wounds and mainly detect the resistance profile to the most common antibiotics used in therapy.

Patients and methods  
This retrospective analysis was conducted by reviewing records of wound swab samples that arrived at the Microbiology laboratory of the ‘Santo Spirito’ Hospital of Pescara in Italy, from March to September 2012. Wounds from diverse aetiologies (predominantly leg ulcers, diabetic foot ulcers and pressure ulcers; surgical wounds were excluded), location and duration progress (acute and chronic) were considered in this study. Information about the gender and age of the patients was also provided. The wounds were sampled for microbiological analysis prior to any administration of antibiotic. In total, 312 wound samples were collected from 213 patients. Some patients had more than one  
wound.

Sample collection  
After superficial precleansing of wounds with physiologic saline, each specimen was collected by rotating a sterile, premoistened swab (Nuova Aptaca SRL, Canelli, Italy) across the wound surface of a 1 cm2 area in a zig-zag motion, from the centre to the outside of the wound. Then, the swab was placed in the tube containing the transport medium (Nuova  
Aptaca SRL) and sent to the Microbiology laboratory of the hospital for further culture analysis.  
Microbiological analysis  
Each swab was plated onto four media: blood agar, McConkey agar, mannitol salt agar and Sabouraud agar (SA). All plates except SA plates were incubated aerobically at 37◦C for 24 hours. SA plates were also incubated aerobically at 30◦C for 48 hours. Any growth was identified by morphologic aspects of the colonies and followed by biochemical identification using the automated Vitek 2 system (bioMerieux, Marcy ´  
l’Etoile, France). The antibiotic susceptibility pattern for each bacterial species previously identified was determined using the Vitek 2 susceptibility testing cards for Gram-positives and Gram-negatives. These procedures followed the manufacturer’s recommendations.

Results  
Overall, 312 wound samples were collected from 213 patients,  
109 (51·2%) were female, and their age ranged from 20 to 100 years. Of all patients, 28 (13·5%) presented at least one relapse and 6 (2·9%) had two or more wounds during the 6-month period considered in the study.In 95 (30·5%) of the wounds only saprophytic bacterial flora was found and there were no signs of infection. The remaining wounds, 217 (69·5%), were considered infected and one or more microbial species with clinical importance were isolated from them. A total of 28 different microbial species were isolated; 44·2% were Gram-positive and 55·8% were Gram-negative. The most common bacterial species detected was Staphylococcus aureus (37%), followed by Pseudomonasaeruginosa (17%), Proteus mirabilis (10%), Escherichia coli(6%) and Corynebacterium spp. (5%) (Figure 1A). The most representative species of Enterococcus was Enterococcuscloacae.  
The presence of only one species isolated from each sample was the most frequent (72·8%). Polymicrobial infection was found in 59 (27·2%) of the infected wounds and was mainly constituted by two species; three species were the maximum number of species isolated per sample and represented only 3**·**4% of the total polymicrobial infections. The predominant species found in polymicrobial infections was S. aureus, P.aeruginosa and P. mirabilis. The most common association was S. aureus/P. aeruginosa (Figure 1B). The antibiotic resistance pattern of the Gram-positive isolates is shown in Table 1. S. aureus and coagulase-negative staphylococci differ significantly in their resistance rate to  
oxicillin, being 21**·**8% and 85**·**7%, respectively. Corynebacterium spp. showed total resistance to oxacillin, as well as to penicillin G, amoxicillin/clavulanic acid, clindamycin, moxifloxacin and levofloxacin. In general, these species showed higher resistance to the majority of the antibiotics tested. The most active agents against all the Gram-positive bacteria tested (with no resistance found) were vancomycin, teicoplanin, linezolid and daptomycin.

Additionally, in Table 2, the antibiotic resistance pattern of the three most common Gram-negatives isolated  
from the wounds is shown. The most effective antibiotic against all these Gram-negatives was amikacin; P.mirabilis and E. coli were fully susceptible to this antibiotic; however, 28**·**3% of P. aeruginosa showed resistance  
to it. The isolates of P. aeruginosa showed 100% of resistance to ampicillin, amoxicillin/clavulanic acid, ertapenem and  
trimethoprim/sulfamethoxazole. Moreover, P. mirabilis and E.coli also showed high resistance to ampicillin. Meropenem,  
ertapenem and fosfomycin were 100% active against E. coli.

**Discussion**  
To prevent or reduce wound infection is a goal shared by  
health care practitioners in charge of wound management and  
care; however, when infection is already established, wound  
management practices should be specifically addressed and  
become more challenging and demanding. Antibiotic treatment is recommended but, previously, an antibiotic susceptibility test should be performed.  
In this study, 28 microbial species were isolated from  
wounds with signs of infection. The majority of the wounds  
were colonised with a single bacterial species. The most common isolate was S. aureus, which was also reported in many  
other studies to be the predominant microorganism (40–60%  
of the total microorganisms) isolated from different types  
of wounds (13–18). P. aeruginosa was the Gram-negative  
more detected, which is also in agreement with other reports  
(Gjødsbøl K, Christensen JJ, Karlsmark T, Jørgensen B, Klein BM,Krogfelt KA. Multiple bacterial species reside in chronic wounds: alongitudinal study.Int Wound J2006;3:225 – 31, Davies CE, Hill KE, Wilson MJ, Stephens P, Hill CM, HardingKG, Thomas DW. Use of 16S ribosomal DNA PCR and denaturinggradient gel electrophoresis for analysis of the microfloras ofhealing and nonhealing chronic venous leg ulcers.J Clin Microbiol2004;42:3549 – 57.,19–21). It is well documented that bacteria such as  
S. aureus and P. aeruginosa produce very destructive virulence factors, responsible for maintaining infection and delay  
healing in chronic wounds. S. aureus causes clinically relevant infections mostly because of its virulence factors such as  
coagulase, catalase, clumping-factor A and leucocidines ( Dissemond J. Methicillin resistantStaphylococcus aureus(MRSA):diagnostic, clinical relevance and therapy.J Dtsch Dermatol Ges2009;6:544 – 51.).  
Similarly, the production of an elastase by P. aeruginosa has  
been associated to its pathogenicity in the wound environment (Schmidtchen A, Holst E, Tapper H, Bjorck L. Elastase-producingPseudomonas aeruginosadegrade plasma proteins and extracellularproducts of human skin and fibroblasts, and inhibit fibroblast growth.Microb Pathog2003;34:47 – 55.). Thus, our results confirm the usual most prevalent  
microorganisms found in infected wounds. However, the role  
that each specific pathogen plays in both no healing and  
infected chronic wounds is not yet very defined; it is mostly  
based on hypotheses (Bowler PG, Duerden BI, Armstrong DG. Wound microbiology andassociated approaches to wound management.Clin Microbiol Rev2001;14:244 – 69., Beldon P. Recognising wound infection.Nurs Times2001;97:3 – 4). Beyond the presence of pathogens,  
it has been considered to be of paramount importance the  
presence of specific bacterial combinations and interactions in both acute and chronic wounds ( Percival SL, Thomas JG, Williams DW. Biofilms and bacterial imbal-ances in chronic wounds: anti-Koch.Int Wound J2010;7:169 – 75.). In our study, only  
27**·**2% of the wounds displayed polymicrobial infections. It is  
known that interspecies interactions consist mostly in bacterial  
synergy that enhances survival, therefore hampering the infection eradication. Moreover, microorganisms have the ability  
to establish themselves and proliferate as a biofilm, both in  
monomicrobial and polymicrobial biofilms, which are often  
considered to be a further complication that has a significant contribution to the lack of successful antibiotic treatment  
(Kirketerp-Møller K, Jensen PØ, Fazli M, Madsen KG, Pedersen J,Moser C, Tolker-Nielsen T, Høiby N, Givskov M, Bjarnsholt T.Distribution, organization, and ecology of bacteria in chronic wounds.J Clin Microbiol2008;46:2717 – 22). Because of this problem, researchers are seeking for new  
alternative therapies useful to enhance wound healing, such as  
laser therapy (Baffoni M, Bessa LJ, Grande R, Di Giulio M, Mongelli M, CiarelliA, Cellini L. Laser irradiation effect onStaphylococcus aureusandPseudomonas aeruginosabiofilms isolated from venous leg ulcer.Int Wound J2012;9:517 – 24).  
The bacterial isolates were examined for their susceptibility pattern to the most commonly used antibiotics in therapy.  
Despite increasing concerns about antibiotic-resistant bacteria, appropriate use of systemic antibiotics is still recommended where there is clear evidence of infection ( Howell-Jones RS, Wilson MJ, Hill KE, Howard AJ, Price PE,Thomas DW. A review of the microbiology, antibiotic usage andresistance in chronic skin wounds.J Antimicrob Chemother2005;55:143 – 9., National Institute for Clinical Excellence.Type 2 diabetes. Preventionand management of foot problems. Clinical guideline 10. London:National Institute for Clinical Excellence, 2004., European Wound Management Association (EWMA).Position doc-ument: management of wound infection. London: MEP Ltd, 2006.).  
The resistance to oxacillin is particularly important because  
it can give us the percentage of methicillin-resistant Staphylococcus aureus (MRSA); in our study, a relevant percentage (21**·**8%) of S. aureus was oxacillin resistant. S. aureushas always been a major source of infection in acute softtissue wounds, but MRSA has only been an infecting organism in a small fraction of the total. Nevertheless, MRSA  
is becoming a more common wound pathogen (Demling RH, Waterhouse B. The increasing problem of woundbacterial burden and infection in acute and chronic soft-tissuewounds caused by methicillin-resistantStaphylococcus aureus.JBurns Wounds2007;7:86 – 98.). The  
occurrence of MRSA presents two problems: the first is  
associated to the chronic wound being a source of other  
MRSA nosocomial infections and the second is related to  
the impact of MRSA on the chronic wound itself, that is,  
who have chronic wounds growing MRSA and have an  
increased risk of suffering a bacteremia by MRSA (Demling RH, Waterhouse B. The increasing problem of woundbacterial burden and infection in acute and chronic soft-tissuewounds caused by methicillin-resistantStaphylococcus aureus.JBurns Wounds2007;7:86 – 98.).  
Among the Gram-positive bacteria, all isolates were susceptible to vancomycin and to linezolid; despite these two antibiotics are largely used, no resistance was found. Regarding  
the antibiotic resistance of Gram-negative, the most common isolates, and in particular P. aeruginosa, showed a  
relatively high resistance to the majority of the antibiotics.  
Multidrug-resistant isolates of P. aeruginosa, that is, fully  
resistant simultaneously to ampicillin, amoxicillin/clavulanic  
acid, ertapenem and trimethoprim/sulfamethoxazole, are of  
major concern. Additionally, the results indicate that P. aeruginosa is tending toward a high level of resistance to carbapenems and third-generation cephalosporins. Similar evidence  
was reported by Nicoletti et al. (30) in a study regarding  
diverse severe infections. P. mirabilis and E. coli showed,  
however, a low-resistance profile compared to P. aeruginosa.  
An important limitation of this study must be mentioned. It  
is evident from the results stated that only aerobic/facultative  
microorganisms were investigated, as the procedures followed  
by the laboratory of the hospital did not perform the isolation  
of anaerobic bacteria from the swab samples collected from  
the wounds. Potential pathogenic anaerobes are frequently  
found in wounds (Brook I, Frazier EH. Aerobic and anaerobic microbiology of chronicvenous ulcers.Int J Dermatol1998;37:426 – 8., Ge Y, MacDonald D, Hait H, Lipsky B, Zasloff M, Holroyd K.Microbiological profile of infected diabetic foot ulcers.Diabet Med2002;19:1032 – 4, rengove NJ, Stacey MC, McGechie DF, Mata S. Qualitativebacteriology and leg ulcer healing.J Wound Care1996;5:277 – 80.). Bowler and Davies (13) reported  
that together with the aerobic and facultative population, the  
diversity of anaerobic bacteria in leg ulcers is considerable  
and their presence was particularly obvious in infected leg  
ulcers in respect to non infected ones. Despite the already  
demonstrated role and importance of anaerobes in wound infection, the techniques to isolate them still not being  
performed in many clinical laboratories in these days; the  
reason seems to be that the culture of anaerobes is more  
time-consuming, labour-intensive and expensive and, thus,  
too demanding. This is certainly a drawback in the wound  
management that should be addressed in order that the best  
treatment will be suggested. Therefore, it is very likely that  
the percentage of polymicrobial infection that we found is  
biased. Gjødsbøl **et al.** (Gjødsbøl K, Christensen JJ, Karlsmark T, Jørgensen B, Klein BM,Krogfelt KA. Multiple bacterial species reside in chronic wounds: alongitudinal study.Int Wound J2006;3:225 – 31.) performed a longitudinal study  
on chronic ulcers and reported that none of these ulcers was  
colonised with only one single bacterial species, but with  
two or more, being the average number of six species per  
ulcer.  
Moreover, it seems to be opportune to state that the  
use of wound swab sampling has been questioned by some  
researchers (Bjarnsholt T, Kirketerp-Møller K, Jensen PØ, Madsen KG, PhippsR, Krogfelt K, Høiby N, Givskov M. Why chronic wounds will notheal: a novel hypothesis.Wound Repair Regen2008;16:2 – 10.) on the basis that it only permits to identify  
the microorganisms present in the surface, neglecting the ones  
present on the deeper tissue; thus, the sample might be lacking  
the correct information of the colonising organisms. However,  
that statement has been opposed by other studies (Davies CE, Hill KE, Newcombe RG, Stephens P, Wilson MJ,Harding KG, Thomas DW. A prospective study of the microbiologyof chronic venous leg ulcers to reevaluate the clinical predictive valueof tissue biopsies and swabs.Wound Repair Regen2007;15:17 – 22, Cooper RA, Ameen H, Price P, McCulloch DA, Harding KG.A clinical investigation into the microbiological status of “locallyinfected” leg ulcers.Int Wound J2009;6:453 – 62) that  
compared different methods to collect wounds samples. In  
these studies, they concluded that the wound swab sampling, if  
appropriate microbiologic culture techniques are used, can be  
an effective method to isolate the microorganisms present. In  
fact, all microbial contaminants (both aerobic and anaerobic)  
are originated from exogenous sources to the wound and,  
consequently, the microorganisms disseminated into deeper  
tissue must also be comprised in the superficial tissue of the  
wound.  
The successful management of bacteria in a wound is  
of great importance; however, it is still a complex issue.  
Therefore, our study evaluates the current situation in a  
particular geographic area, which is mostly helpful to the  
clinicians and microbiologists involved because it can make  
them aware of the real circumstances that they are dealing  
with presently. Knowing the prevalent type of microorganisms  
present in infected wounds and their resistance pattern is  
clearly pertinent to choose the adequate treatment. The  
data presented here together with the discussion carried  
out can be useful to improve the management of wound  
infection.