International Scholars Journals

International Journal of Virology and Parasitology, Vol. 11 (2), pp. 001-015, February, 2022. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Molecular identification of microorganisms in chronic wounds, Republic of Guinea (Conakry)

Cyrille Bilé Ehounoud[1,2], Alpha Kabinet Keita[3], Abdoul Habib Béavogui [4,5], Ansoumane Cissé[4], Nadia Amanzougaghene[1], Jean David N'Guessan[2], Didier Raoult[1], Oleg Mediannikov[1], Florence Fenollar[1]*

[1]Aix Marseille Univ, Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes (URMITE), UM63, CNRS 7278, IRD 198, INSERM 1095, IHU - Méditerranée Infection, 19-21 Boulevard Jean Moulin, 13005 Marseille

[2]Felix Houphouet Boigny Université, UFR Biosciences, Côte D'Ivoire.

[3]Unité de recherche translationnelle appliquée au VIH et les maladies infectieuses (TransVIHMI) IRD UMI 233-INSERM U 1175 Centre IRD France Sud 911, avenue Acropoles, BP 64501 F-34394 Montpellier cedex 5.

[4]Centre de Formation et de Recherche en Santé Rurale de Mafèrinyah B.P. 2649, Conakry, République de Guinée.

[5]Faculté de Médecine, Pharmacie et Odonto-Stomatologie, Université Gamal Abdel Nasser de Conakry, République de Guinée.

Accepted 13 January, 2022

Skin infections are common in sub-Saharan Africa, including chronic wounds. This study aimed to assess the presence of several microorganisms in skin specimens from patients with chronic wounds and healthy people in Maferinyah (Republic of Guinea). Eighty-four skin samples from the wounds of 20 patients (42 edge swabs and 42 center swabs) and twenty-two skin samples from 11 healthy people were analyzed by real-time quantitative PCR and standard PCR assays combined with sequencing. *Pseudomonas aeruginosa* was the most frequently detected bacterium, which was significantly more prevalent in patients (80%, 16/20) than in healthy people (9%, 1/11; *p*<0.001), followed by *Staphylococcus aureus* which was only detected in patients (60%, 12/20; *p*<0.001). *Streptococcus pyogenes* was also more frequently detected in patients (30%, 6/20) than in healthy people (9%, 1/11) but the difference was not statistical significant. *Rickettsia felis* was also detected for the first time in Guinea, in one patient. Finally, species of the genus *Acinetobacter* were also frequently and exclusively detected in patients (80%, 16/20). *Acinetobacter baumannii* (2/20, 10%), *Acinetobacter nosocomialis* (10%), *Acinetobacter junii* (1/20, 5%), *Acinetobacter lwofii* (5%), and *Acinetobacter guangdongensis* (5%), which was detected for the first time in skin, were identified. *Acinetobacter junii* and *Acinetobacter lwofii* were observed in different samples from the same patient. For the 11 other patients, polymicrobial infections featuring several species of the genus *Acinetobacter* were observed. Overall, many different bacteria which may encourage wound enlargement or delayed healing were observed in chronic wounds.

Keywords: skin; chronic wound; bacteria; *Staphylococcus aureus*; *Pseudomonas aeruginosa*; *Acinetobacter* spp.; *Rickettsia felis*; Guinea (Conakry)

INTRODUCTION

Chronic wounds represent a common pathology in poor countries, affecting about 15% of the population in Sub-Saharan Africa compared to 1% in developed countries (Gulam-Abbas et al., 2002; Gottrup, 2004). Diabetic foot ulcers, venous leg ulcers, surgical wounds, eschars, burns, and bites are regularly observed (Rhoads et al., 2012; Mediannikov et al., 2014; Essayagh et al., 2014; Pratt et al., 2016). Chronic wounds have a real impact upon morbidity and disability, as patients can live for years with wounds, but also have an impact upon mortality. In a study on diabetic foot ulcers in Tanzania (Sub-Saharan Africa), Gulam-Abbas *et al.* reported that the mortality rate was up to 54% among in-patients with severe ulcerations without amputation of the affected part.1 Smith also speculate that in future years, the death rate will increase because of the high costs of care (Smith, 2004), which may often force patients to treat themselves at home or to consult alternative medicine providers. Studies on foot ulcers estimate that amputation rates are 45% and 23.5% and mortality is 38% and 9%, respectively, in western and northern African countries (Sano et al., 1998; Benotmane et al., 2000). These prevalence rates demonstrate the extent of the problem of chronic wounds in which infections represent a major cause of amputations and deaths (Gomez et al., 2009; Sen et al., 2009; Wolcott et al., 2010a).

Most wounds infections are caused by bacteria. The most common are *Staphylococcus aureus, Streptococcus pyogenes*, and *Pseudomonas aeruginosa* (Gjødsbøl et al., 2006; Rhoads et al., 2012; Mediannikov et al., 2014). The presence of *Acinetobacter* spp., including *Acinetobacter baumannii*, is also significant in burn wound infections.

One study reported that they were the most prevalent (22.2%) followed by *P. aeruginosa* (15.1%), and *S. aureus* at 10.3% (Essayagh et al., 2014). *Rickettsia felis*, a bacterium involved in fever in sub-Saharan Africa and Asia was reported in eschars (7.4%) but also in skin from healthy people (5%) in Senegal (Mediannikov et al., 2014; Socolovschi et al., 2010; Ferdouse et al., 2015; Mourembou et al., 2015a).

Molecular testing methods present several advantages, such as the ability to identify fastidious bacteria and dead bacteria following, for example, antibiotic therapies or when specimens have been kept in poor transport or storage conditions. Thus, although molecular methods cannot replace culture in terms of obtaining isolates and data about antibiotic susceptibilities, they provide a significant amount of information and enable the description of bacterial repertoire (Thomsen et al., 2010; Wolcott et al., 2010b; Rhoads et al., 2012).

Buruli ulcer is another cause of skin disease associated with tropical and humid areas in Africa where there are slow moving rivers and stagnant water (Wansbrough-Jones and Phillips, 2006). This chronic skin disease is caused by infection with *Mycobacterium ulcerans* leading to the development of large ulcers (Wansbrough-Jones and Phillips, 2006). Environmental sources of *M. ulcerans* are better characterized but the mode of transmission of infection is still uncertain (Wansbrough-Jones and Phillips, 2006). The introduction of rational antibiotic therapy has resulted in improvements in the management of the disease (Wansbrough-Jones and Phillips, 2006).

In Guinea, little is known about the microorganisms present in chronic wounds. This study aimed to evaluate the prevalence of microorganisms in chonic wounds in Guinea (Conakry), including the use of controls (healthy skin), as new strategies have emerged indicating the possible role of a microorganism as the cause of infection.

PATIENTS, MATERIAL AND METHODS

Patients and control group

This study includes 84 skin samples obtained from 20 patients with chronic skin wounds. These patients, who live in rural areas, have consulted at Primary Health Care center of Maferinyah, Republic of Guinea (Conakry) in June 2014. At the same time, 22 skin specimens from 11 healthy people living in the same area were sampled (Table 1). Samples were collected according the protocol previously reported by Mediannikov *et al* (Mediannikov et al., 2014). Briefly, the cotton swab (Copan, Brescia, Italy) was applied firmly to the center and edge of the wound. For negative control samples, the cotton swab was applied to the skin (inner surface of the forearm) of healthy people. All lesions were photographed. All samples were transferred to the URMITE laboratory (Marseille, France).

The information gathered on each patient included their age, sex, the presence of fever (axillary temperature > 37.5°C), glycemia (0.75 g/L < normal glycemia < 1.10 g/L), history, evolution of the wound, and use of river water. None of the patients had received antibiotic treatment or local antiseptic treatment before sampling.

Ethics Statement

This study was approved by the ethics committee of Guinea, Conakry (agreement number 008/CNERS/14). Written informed consent from all participants, including patients and the parents or legal guardians of children was obtained.

*Corresponding Author's Email: florence.fenollar@univ-amu.fr

Table 1. Distribution of samples according to sample area.

		Sample area		
		Edge	Center	
	People	Number of samples		Total
Wounds	20	42	42	84
Healthy skin	11	11	11	22

one-second cycles at 95°C, 35 seconds at 60°C, and

Molecular analysis

Each cotton swab was put in 200 µL of buffered solution (G2; Qiagen, Hilden, Germany) with 20 µL of proteinase K (Qiagen) and incubated at 56°C for one hour. DNA from each sample was extracted using the EZ1 DNA Tissue kit following the manufacturer's recommendations (Qiagen). The quality of all DNA extracts was checked using quantitative real-time PCR (qPCR) targeting a specific human β-actin gene (primers ActinF 5'-CATGCCATCCTGCATCTGGA-3' and ActinR 5'-CCGTGGCCATCTCTTGCTCG-3' combined with a TaqMan probe 6-FAM-CGGGAAATCGTGCGTGACATTAAG-TAMRA), as previously reported (Keita et al., 2015; Mourembou et al., 2015b).

Pathogen screening of skin samples was performed with qPCR assays using primers and probes targeting *S. aureus, S. pyogenes, Streptococcus pneumoniae, Salmonella* spp., *Acinetobacter* spp.*, P. aeruginosa*, *Tropheryma whipplei, Rickettsia* spp., *R. felis*, *Mycobacterium* spp., *Mycobacterium ulcerans, Mycobacterium marinum, Coxiella burnetii, Treponema pallidum, Haemophilus ducreyi*, *Leishmania* spp., *Mansonella* spp., and Pox Virus (Table 2) (Rolain et al., 2002; Rolain et al., 2005; Leslie et al., 2007; Mediannikov et al., 2010; Bouvresse et al., 2011; Guitard et al., 2012; Lavender et al., 2012; Hamad et al., 2015; Mourembou et al., 2015a; Mourembou et al., 2015b; Mourembou et al., 2016).

Each PCR assay was performed with a 20 µL volume containing 10 µL Master mix No-ROX (Eurogentec, Liege, Belgium), 3.5 µL of distilled water (DNAase /RNAase free), 2.5 µM of probe, 20 µM of each primer, and 5 µL of DNA extract (Keita et al., 2015; Mourembou et al, 2015b). All reactions were performed using a CFX 96 (Bio-Rad, Marnes-la-Coquette, France) according to the manufacturer's protocol: DNA denaturation steps at 50°C for two minutes and 95°C for five minutes followed by 40

extension steps for 30 seconds at 45°C. In each reaction, two positive controls (microbial DNA) and two negative controls (the mix alone) were used to validate each PCR assay.

For the identification of *Acinetobacter* species, DNA extracts were subjected to standard PCR to amplify a portion of the *rpoB* gene, coupled with sequencing. The primers used (Ac696 Forward TAYCGYAAAGAYTTGAAAGAAG and Ac1093 Reverse CMACACCYTTGTTMCCRTGA) amplified a 350 bp fragment of *Acinetobacter rpoB* gene, as previously reported (La Scola et al., 2006). Standard PCR was performed on a ThermalCycler (Applied Biosystem, Paris, France). The reactions were carried out using the Hotstar Taq-polymerase (Qiagen), in accordance with the manufacturer's instructions. The amplicons were visualized using electrophoresis on a 1.5% agarose gel stained with ethidium bromide and examined using an ultraviolet transilluminator. The PCR products were purified using a PCR filter plate Millipore NucleoFast 96 PCR kit following the manufacturer's recommendations (Macherey-Nagel, Düren, Germany) (Ehounoud et al., 2016). The amplicons were sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) with an ABI automated sequencer (Applied Biosystems). The obtained sequences were assembled using ChromasPro software (ChromasPro 1.7, Technelysium Pty Ltd and Tewantin, Australia) and compared with those available in GenBank by NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Ehounoud et al., 2016).

A phylogenetic tree was constructed by using the test maximum likelihood in the MEGA6 program (http://megasoftware.net/). The Epi Info version 7 program (http://www.cdc.gov/epiinfo/index.html) was used for data analysis. A difference was statistically significant when *p*-values were <0.05.

Table 2. Primers and probes used for real-time quantitative PCR in this study.

Microorganisms detected	Targeted sequences	Primers (5'-3') Forward Reverse Probes (6 FAM – TAMRA)	References
BACTERIA			
Coxiella burnetii	*IS1111*	CAAGAAACGTATCGCTGTGGC CACAGAGCCACCGTATGAATC CCGAGTTCGAAACAATGAGGGCTG	(Rolain et al., 2005)
	Hypothetical Protein	CGCTGACCTACAGAAATATGTCC GGGGTAAGTAAATAATACCTTCTGG CATGAAGCGATTTATCAATACGTGTATGC	(Mediannikov et al., 2010)
Haemophilus ducreyi	*GroESL*	CACAATGAGTATTCGTCCATTACAC GCAATCACTTTACCGCGAGT CGGGTGGTATTGTTTTAACAGGTTCAGCGA	This study
Mycobacteria	ITS	GGGTGGGGTGTGGTGTTTGA CAAGGCATCCACCATGCGC TGGATAGTGGTTGCGAGCATC	(Guitard et al., 2012)
Mycobacterium ulcerans	IS2404	AAAGCACCACGCAGCATCT AGCGACCCCAGTGGATTG CGTCCAACGCGATC	(Lavender et al., 2012)
Mycobacterium marinum	*Ppe*	ATGTGGGCAGCTTCAATGTG CCAAGCCAACACTGGAATCA AACATCGGGCCGGGCAACCT	This study
Pseudomonas aeruginosa	*OprL*	CGCTGCCTTTCAGGTCTTTC CGTGCGATCACCACCTTCTA TCCAGAGCGCGCATGGCTTC	This study
	Hypothetical Protein	GAACCGTTGTGCAGGTAGGG CGCAAGGACTACTGCCTGAA CGGTGGCCCAGATGCCGTTC	This study
***Rickettsia* spp.**	RKNDO3	GTGAATGAAAGATTACACTATTTAT GTATCTTAGCAATCATTCTAATAGC CTATTATGCTTGCGGCTGTCGGTTC	(Rolain et al., 2002)
Rickettsia felis	0527	ATGTTCGGGCTTCCGGTATG CCGATTCAGCAGGTTCTTCAA GCTGCGGCGGTATTTTAGGAATGGG	(Mourembou et al., 2015a)
	OrfB	CCCTTTTCGTAACGCTTTGCT GGGCTAAACCAGGGAAACCT TGTTCCGGTTTTAACGGCAGATACCCA	(Mourembou et al., 2015a)

Table 2. Continue

***Salmonella* spp.**	*SipC*	GTCAGGCGTCGTAAAAGCTG ACGTCGACTGGTGGTACTGG CTCCAGGCGCGAACAGCTGG	(Mourembou et al., 2016)
	InvA	TCTGTTTACCGGGCATACCA CACCGTGGTCCAGTTTATCG CCAGAGAAAATCGGGCCGCG	(Mourembou et al., 2016)
Streptococcus pneumoniae	*PlyN*	GCGATAGCTTTCTCCAAGTGG TTAGCCAACAAATCGTTTACCG CCCAGCAATTCAAGTGTTCGCCGA	(Mourembou et al., 2016)
Streptococcus pyogenes	Hypothetical Protein	ACAGGAACTAATACTGATTGGAAAGG TGTAAAGTGAAAATAGCAGCTCTAGCA AAAATGTTGTGTTTTAGGCACTGGCGG	(Mourembou et al., 2016)
	MipB	GGACATAATAAAAGGTTTTTCTTCCA CAAAATACACAAAATACAGAACCAAA CATTATGATGTGACGTGGTAGGATGGG	(Mourembou et al., 2016)
Staphylococcus aureus	*NucA*	TTGATACGCCAGAAACGGTG TGATGCTTCTTTGCCAAATGG AACCGAATACGCCTGTAC	(Mourembou et al., 2016)
	Amidohydrolase	CCTCGACAGGTAACGCATCA AAACTCCTATCGGCCGCAAT TGCAATGGTAGGTCCTGTGCCCA	(Mourembou et al., 2016)
Tropheryma whipplei	*whi2*	TGAGGATGTATCTGTGTATGGGACA TCCTGTTACAAGCAGTACAAAACAAA GAGAGATGGGGTGCAGGACAGGG	(Keita et al., 2015)
	whi3	TTGTGTATTTGGTATTAGATGAAACAG CCCTACAATATGAAACAGCCTTTG GGGATAGAGCAGGAGGTGTCTGTCTGG	(Keita et al., 2015)
***Acinetobacter* spp.**	*rpoB*	TACTCATATACCGAAAAGAAACGG GGYTTACCAAGRCTATACTCAAC CGCGAAGATATCGGTCTSCAAGC	(Bouvresse et al., 2011)
Acinetobacter baumannii	*Nacetyl glutamate synthase*	ARCGGATGCCAAGAGAATGT CCGACATTCAGCACCCTACA GCGGACTGCTTCACCGCCAA	This study
	pap	AAAAAGAGCGTGCACGACAA TCGGCCCAAAAATAACTTGG GCGCAAGCGGGTACAACGTGA	This study

Table 2. Continue

Treponema pallidum	*polA*	GTCGAGACTGAAAAGGAGTGCA GTGAGCGTCTCATCATTCCAAAG TGCTGTGCAGGATCCGGCATATGTCC	(Leslie et al., 2007)
PARASITES			
***Leishmania* spp**	*18S*	ACAAGTGCTTTCCCATCG CCTAGAGGCCGTGAGTTG CGGTTCGGTGTGTGGCGCC	(Hamad et al., 2011)
***Mansonella* spp**	*ITS*	CCTGCGGAAGGATCATTAAC ATCGACGGTTTAGGCGATAA CGGTGATATTCGTTGGTGTCT	(Mourembou et al., 2015b)
VIRUS			
Pox Virus	*Hemagglutinin*	TGATGCAACTCTATCATGTARTCG CAAGACGTCGCTTTTRGCAG TGCTTGGTATAAGGAGCCCAATTCCA	This study
	B2L	CGGTGCAGCACGAGGTC CGGCGTATTCTTCTCGGACT GCCTAGGAAGCGCTCCGGCG	This study

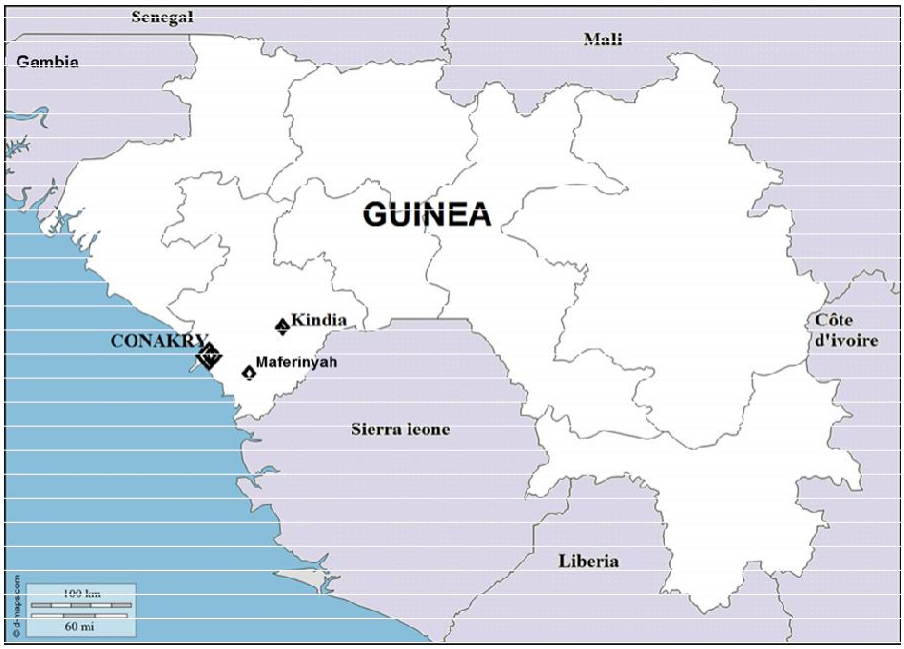


Figure 1. Map of Guinea (Conakry) showing the area of Maferinyah where people were recruited.

RESULTS

Description of the population

Twenty patients (8 males and 12 females) with chronic wounds, residing in the rural area of Maferinyah, which is crossed by the Kili river, were included (Figure 1). Their

ages ranged from 13 to 90 year-old (mean age 47). Of them, six patients (6/20; 30%) presented a fever and eight (8/20; 40%) presented hyperglycemia (glycemia ≥ 1.10 g/L). Eleven patients (11/20; 55%) used the water from river to wash their bodies, kitchenware, and linen.

Patients had presented wounds from between three weeks and 19 years. Most of the wounds (16/20; 80%)

Table 3. Main epidemiological, biological, and clinical data of patients with chronic wounds.

Patients	Data					
	Temperature (≥37.5°)	Glycemia (≥1.10)	History of wound	Evolution of wound	Localization of wound	Use of river water for their needs
1	no	no	Swelling	2 years	External malleolus	yes
2	no	yes	Minor injury	NA	Back foot	yes
3	yes	no	Trauma object	2 years	Back foot	no
4	no	no	Minor injury	3 years	External malleolus	no
5	no	yes	Sharp object	10 years	Above the ankle	yes
6	no	no	Swelling	5 months	Back foot	no
7	no	no	Swelling	4 months	External malleolus	yes
8	no	no	Swelling	5 years	Back foot	yes
9	no	no	Minor injury	3 weeks	Back foot	no
10	no	yes	Snakebite	4 years	Back foot	yes
11	yes	no	Wood	7 years	Back foot	no
12	yes	no	Snakebite	2 years	Above the ankle	NA
13	no	yes	Swelling	19 years	Back foot	yes
14	yes	yes	Palm thorn	3 years	Back foot	yes
15	yes	yes	Minor injury	8 months	External malleolus	yes
16	no	no	Falling tree	1 year	Above the ankle	yes
17	no	no	Snakebite	6 years	Back foot	no
18	no	no	Sharp object	5 years	Sole	no
19	no	yes	NA	6 months	Above the ankle	no
20	yes	yes	NA	3 months	Foot / Above the ankle	yes

NA: Not available

were located on the foot, such as the back of the foot for 11 patients, external malleolus for four patients, and the sole in one case. For the remaining five patients (5/20; 25%), wounds were located above the ankle. The wounds had been caused by a sharp object for four patients (4/20; 20%), by a snake bite for three patients (3/20; 15%), by a scratch for two patients (2/20; 10%), and by other means for the last 11 patients (Table 3).

Microorganisms detected

Because ß-actin qPCR was positive for all samples, revealing the good quality of DNA extracts, all specimens were included in the analyses. All the patients as well as two of the 11 healthy people were positive for at least one bacterium (Table 4). All the detected bacteria are summarized in Table 5.

P. aeruginosa was the most frequently observed microorganism in both patients (80%, 16/20) and healthy people (9%, 1/11). However, the bacterium was statistically more frequently observed among patients than the control group with healthy skin (80% versus 9%, $p<0.001$). Overall, *P. aeruginosa* was observed in 56 of the 84 skin samples taken from patients (66.6%) and in one of the 22 skin samples taken from healthy people (4.5%). *P. aeruginosa* was also detected at approximately the same prevalence at the edges (69%, 29/42) and centers (64.2%, 27/42; $p=0.6$) of the wounds.

S. aureus was the second more common microorganism identified among patients (60%, 12/20). *S. aureus* was also statistically more frequently detected among patients, as it was not detected in any skin samples taken from healthy people (0% versus 60%, $p<0.001$). In addition, *S. aureus* was observed in 29 of the 84 skin samples taken from patients (34.5%). Overall, *S. aureus* was more frequently detected at the edges of wounds (40.4%, 17/42) than in the centers (28.5%, 12/42), although the difference was not statistically significant ($p=0.3$).

S. pyogenes was more frequently observed in patients (30%, 6/20) than in healthy people (9%, 1/11) but the difference was not statistically significant ($p=0.1$). Overall, the bacterium was found in 15 of the 84 skin samples taken from patients (17.8%) and in one of the 22 healthy skin samples (4.5%) but there was no significant statistical

Table 4. Microorganisms identified in skin samples for each patient and healthy people (**Acinetobacter* **sp P signifies that several species of** ***Acinetobacter*** **were present in the specimens).**

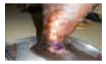
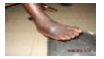
People	Detected microorganisms			
	Center swab	**Edge swab**	**Total**	**Wounds**
Patient 1	*P. aeruginosa* + *"Acinetobacter* sp P*"	*P. aeruginosa*	*P. aeruginosa* + *"Acinetobacter* sp P"	
Patient 2	*P. aeruginosa* + *S. aureus*	*P. aeruginosa*	*P. aeruginosa* + *S. aureus*	
Patient 3	*P. aeruginosa* + *"Acinetobacter* sp P"	*P. aeruginosa* + *"Acinetobacter* spP" + *S. aureus*	*P. aeruginosa* + *"Acinetobacter* sp P" + *S. aureus*	
Patient 4	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	
Patient 5	*P. aeruginosa* + *S. aureus*	*P. aeruginosa* + *S. aureus*	*P. aeruginosa* + *S. aureus*	
Patient 6	*P. aeruginosa* + *"Acinetobacter* sp P"	*P. aeruginosa* +*"Acinetobacter* sp P" + *S. pyogenes*	*P. aeruginosa* + *"Acinetobacter* sp P" + *S. pyogenes*	
Patient 7	*P. aeruginosa* + *"Acinetobacter* sp P"	*P. aeruginosa* + *Acinetobacter* spp	*P. aeruginosa* + *"Acinetobacter* sp P"	
Patient 8	*S. aureus* + *S. pyogenes*	*S. pyogenes* + *"Acinetobacter* sp P"	*S. aureus* + *S. pyogenes* + *"Acinetobacter* sp P"	
Patient 9	*P. aeruginosa* + *A. baumannii*	*P. aeruginosa* + *A. baumannii* *S. aureus* + *S pyogenes*	*P. aeruginosa* + *A. baumannii* *S. aureus* + *S. pyogenes*	
Patient 10	*P. aeruginosa* + *R. felis* + *"Acinetobacter* sp P"	*P. aeruginosa* + *"Acinetobacter* sp P"	*P. aeruginosa* + *R. felis* + *"Acinetobacter* sp P"	
Patient 11	*S. pyogenes*	*S. aureus* + *S. pyogenes* + *A. nosocomialis*	*S. aureus* + *S. pyogenes* + *A. nosocomialis*	
Patient 12	*P. aeruginosa*	*P. aeruginosa* + *S. aureus*	*P. aeruginosa* + *S. aureus*	
Patient 13	*P. aeruginosa* + *S. aureus* + *A. nosocomialis*	*P. aeruginosa* + *S. aureus*	*P. aeruginosa* + *S. aureus* + *A. nosocomialis*	
Patient 14	*A. baumannii*	*A. baumannii*	*A. baumannii*	
Patient 15	*P. aeruginosa* + *"Acinetobacter* sp P"	*"Acinetobacter* sp P"	*P. aeruginosa* + *"Acinetobacter* sp P"	

Table 4. Continue

Patient 16	*P. aeruginosa* + *S. aureus* + *"Acinetobacter* sp P*"*	*P. aeruginosa* + *S. aureus* + *"Acinetobacter* sp P*"*	*P. aeruginosa* + *S. aureus* + *"Acinetobacter* sp P*"*	
Patient 17	*P. aeruginosa* + *S. aureus* + *A. guangdongensis*	*P. aeruginosa* + *S. aureus* + *A. guangdongensis*	*P. aeruginosa* + *S. aureus* + *A. guangdongensis*	
Patient 18	*P. aeruginosa* + *S. aureus* + *"Acinetobacter* sp P*"*	*P. aeruginosa* + *S. aureus* + *"Acinetobacter* sp P*"*	*P. aeruginosa* + *S. aureus* + *"Acinetobacter* sp P*"*	
Patient 19	*S. pyogenes* + *"Acinetobacter* sp P*"*	*S. pyogenes* + *Acinetobacter* spp	*S. pyogenes* + *"Acinetobacter* sp P*"*	
Patient 20	*S. aureus* + *A. lwoffii*	*P. aeruginosa* + *S. pyogenes* + *A. junii*	*P. aeruginosa* + *S. aureus* + *S. pyogenes*+ *A. junii* + *A. lwoffii*	
Healthy people 1	None	*P. aeruginosa*	*P. aeruginosa*	None
Healthy people 2	None	None	None	None
Healthy people 3	None	None	None	None
Healthy people 4	None	None	None	None
Healthy people 5	None	None	None	None
Healthy people 6	None	None	None	None
Healthy people 7	None	None	None	None
Healthy people 8	None	None	None	None
Healthy people 9	None	None	None	None
Healthy people 10	None	*S. pyogenes*	*S. pyogenes*	None
Healthy people 11	None	None	None	None

(55.9%). For twenty-one patients (21/47; 44.6%), the species of *Acinetobacter* was successfully identified. Of all the species of *Acinetobacter* identified, *A. baumannii* was detected in two patients (2/20, 10% versus 0% in healthy people; p=0.4). For the two patients, the similarity was 100% with *A. baumannii,* previously detected in tissue in Germany (Genbank LN868200). Overall, *A. baumannii* was observed in seven of the 84 specimens (8.3%). Of them, 9.5% (4/42) were detected from the edges of wounds and 7.1% (3/42; p=0.5) from the centers.

Acinetobacter nosocomialis was detected in two other patients (10%, 2/20 versus 0% in healthy people; p=0.4). There was 100% similarity with the *A. nosocomialis* strain A196

difference (p=0.1). The edges of wounds were more often tested positive for *S. pyogenes* (23.8%, 10/42) than the centers (11.9%, 5/42) but the difference was not statistically significant (p=0.1). *R. felis* was detected in only one patient (5%, 1/20) but not in the skin of healthy people (p=0.6). In addition, the bacterium was found in one sample taken from the edge of the wound (1.1%, 1/84).

Acinetobacter spp. was only identified in patients (80%, 16/20) and was not found in the skin samples taken from healthy people (0%, 0/11; p<0.001). *Acinetobacter* spp. was found in 47 of the 84 skin samples taken from patients

(KJ788897) for one patient and the LMG10619 strain (LC102686) for the other. *A. nosocomialis* was observed in 7.1% (6/84) of the specimens: 9.5% (4/42) from the edges of wounds and 4.7% (2/42; p=0.3) from the center.

Acinetobacter guangdongensis was identified in one patient (1/20, 5% versus 0% in healthy people; p=0.6). There was 99% of similarity with the *A. guangdongensis* strain ANC5077 (KR611818.1). Overall, *A. guangdongensis* was detected in 4.7% (4/84) of the samples: two from the edges and two from the center of the wound.

Table 5. Prevalence of microorganism in patients and healthy people including samples (**Acinetobacter* **sp P signifies that several species of** ***Acinetobacter*** **were present in the specimens).**

Microorganisms	Patients Percentage (Number of positive/Number of tested)	Healthy people	*p-value* (Patients/Healthy people)	Samples Wound edges	Samples Wound centers	Total wounds (edge / center)	Samples Healthy skins	*p-value* (Wounds / Healthy skins)
Pseudomonas aeruginosa	80% (16/20)	9% (1/11)	< 0.001	64.2% (27/42)	69 % (29/42)	66.6 % (56/84)	4.5% (1/22)	< 0.001
Staphylococcus aureus	60% (12/20)	0% (0/11)	< 0.001	40.4 % (17/42)	28.5 % (12/42)	34.5 % (29/84)	0% (0/22)	< 0.001
Streptococcus pyogenes	30% (6/20)	9% (1/11)	0.1	23.8 % (10/42)	11.9 % (5/42)	17.8% (15/84)	4.5% (1/22)	0.06
***Acinetobacter* spp.**	80% (16/20)	0% (0/11)	< 0.001	61.9% (26//42)	50% (21//42)	55.9 % (47/84)	0% (0/22)	< 0.001
***"Acinetobacter* sp P"**	55% (11/20)	0% (0/11)	< 0.001	35.7% (15/42)	28.5% (12/42)	32.1% (27/84)	0% (0/22)	< 0.001
Acinetobacter baumannii	10% (2/20)	0% (0/11)	0.2	7.1% (3/42)	9.5% (4/42)	8.3 % (7/84)	0% (0/22)	0.09
Acinetobacter nosocomialis	10% (2/20)	0% (0/11)	0.2	9.5% (4/42)	2.3% (1/42)	5.9 % (5/84)	0% (0/22)	0.1
Acinetobacter guangdongensis	5% (1/20)	0% (0/11)	0.3	4.7% (2/42)	4.7% (2/42)	4.7% (4/84)	0% (0/22)	0.1
Acinetobacter junii	5% (1/20)	0% (0/11)	0.3	4.7% (2/42)	0% (0/42)	2.3 % (2/84)	0% (0/22)	0.3
Acinetobacter lwoffii	5% (1/20)	0% (0/11)	0.3	0% (0/42)	4.7% (2/42)	2.3 % (2/84)	0% (0/22)	0.3
Rickettsia felis	5% (1/20)	0% (0/11)	0.3	0% (0/42)	2.3% (1/42)	1.9 % (1/84)	0% (0/22)	0.3

Acinetobacter junii and *Acinetobacter lwoffii* were identified in the same patient (5%, 1/20 versus 0%; p=0.6). There was 99% similarity with the *A. junii* strain NBRC109759 (LC102684) and 100% similarity with the *A. lwoffii* strain LMG1300 (EF611398). *A. junii* was detected in two of the 42 specimens (4.7%) from the edges of wounds. *A. lwoffii* was detected in two of the 42 specimens (4.7%) from the center of wounds (Figure 2).

Identification failed for the 26 others samples which were tested positive for *Acinetobacter* spp.

A mixed electropherogram indicating polymicrobial infection with several *Acinetobacter* species was observed for all these samples (Figure 3). Polymicrobial *Acinetobacter* infection was observed in 11 of the 20 patients (55%), marking a significant difference compared to healthy people (0%, 0/11; p<0.001).

S. pneumoniae, *C. burnetii, Salmonella* spp., *Mycobacteria* spp., *H. ducreyi*, *Leishmania* spp*.,* *Mansonella* spp*., T. pallidum*, and *Poxvirus* were not detected in either wounds or healthy skin.

DISCUSSION

The skin is mainly colonized by non-pathogenic bacterial flora. In healthy people, some pathogenic bacteria, particularly *S. aureus,* can colonize the skin without clinical manifestations (Wertheim et al., 2005). Asymptomatically, *S. aureus* colonization is estimated to affect approximately 30% of the human population (Tong et al., 2015). In addition, *S. aureus* can cause various diseases, including skin and soft tissue infections, particularly when skin or mucosal barriers have been breached (Tong et al., 2015). Indeed, rupture of the skin barrier is the primary factor promoting infection (Scales and Huffnagle, 2013) allowing microorganisms to enter,

multiply, and spread within the body. It is

also reported that wound chronicity is not associated with a single species of bacteria but rather to a polymicrobial biofilm formed by bacteria (Dowd et al., 2008; Percival et al., 2010).

In this study, we evaluate the prevalence of several microorganisms in skin samples taken from people with chronic wounds and healthy people, using molecular methods in Guinea (Conakry), Africa. Our data are consistent as they are based on rigorous interpretation criteria. The quality of DNA extracts was systematically checked and each PCR assay was also systematically validated by the presence of positive and negative controls. In addition, each sample which tested positive for a microorganism with an initial PCR assay was systematically confirmed by a second PCR assay targeting a sequence other than that previously tested.

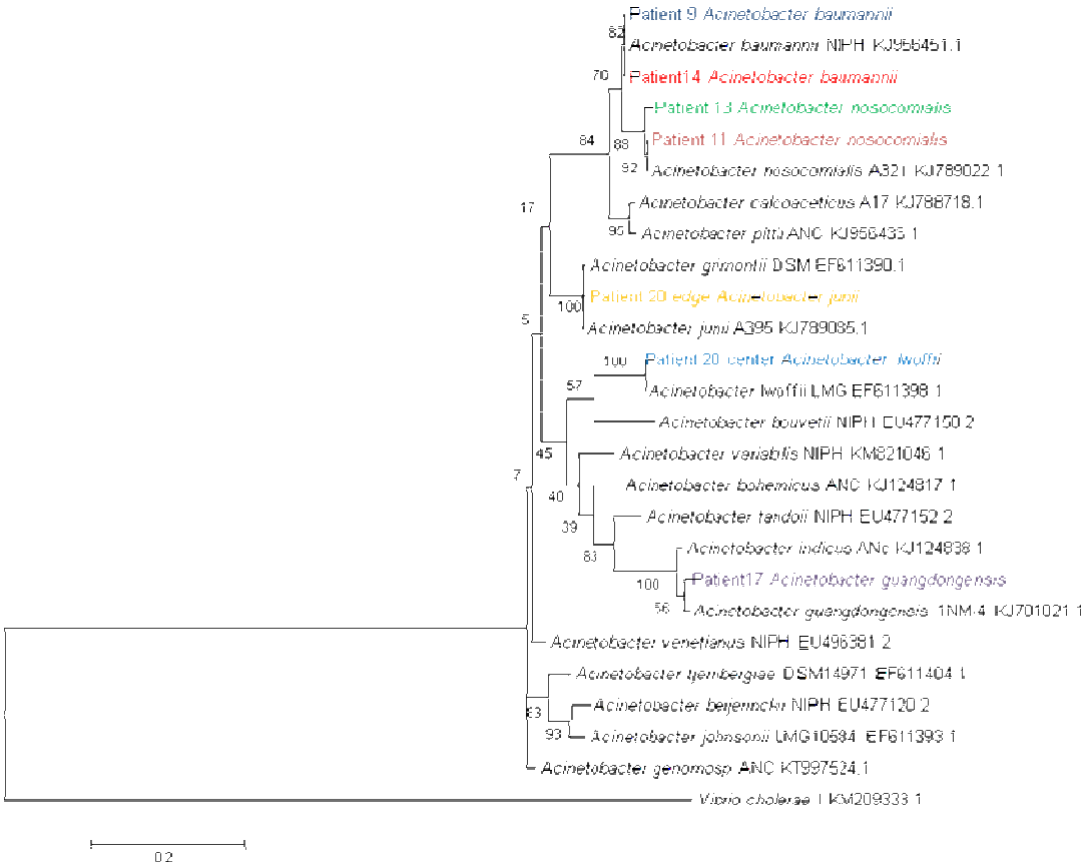


Figure 2. Phylogenetic tree highlighting the position of *Acinetobacter* species identified in the study. The *Rpo B* sequences were aligned using MEGA 6 and phylogenetic inferences were obtained using the maximum likelihood standard method.

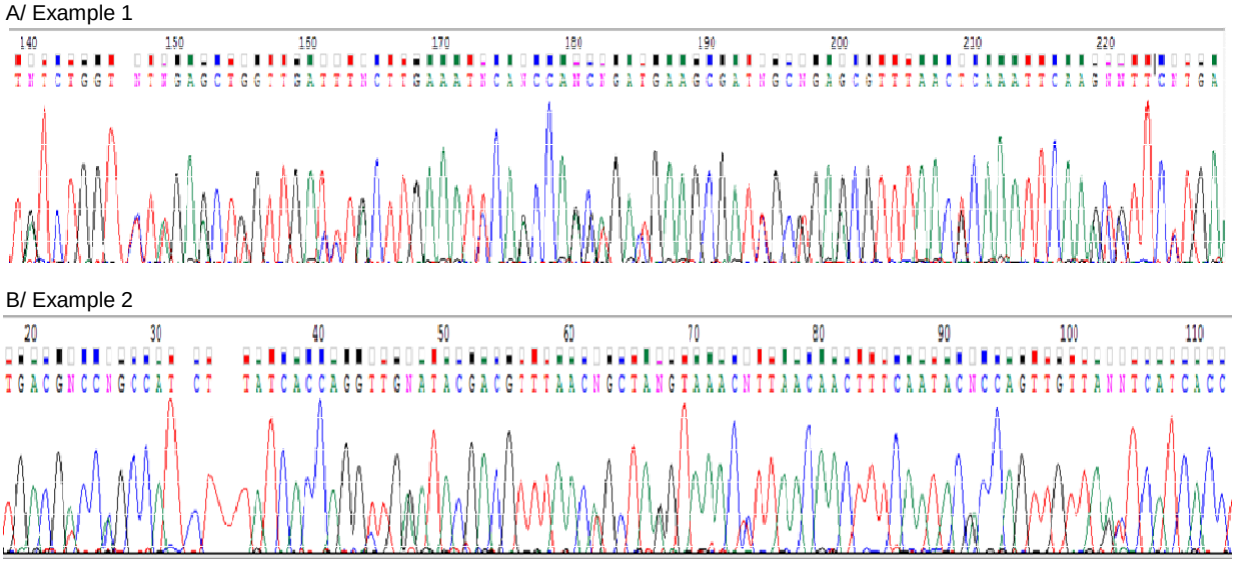


Figure 3. Two examples of mixed electropherogram observed in the study. They indicate polymicrobial infection with several *Acinetobacter* species.

(Parola et al., 2015; Angelakis et al., 2016). If this is the

As previously reported, *S. aureus* and *P. aeruginosa* were the most common bacteria identified in chronic wounds, with a significant difference compared to healthy skin (Gjødsbøl et al., 2006; Rhoads et al., 2012; Serra et al., 2015). Each of these bacteria can express virulence factors and surface proteins that may affect wound healing (Serra et al., 2015; Bessa et al., 2015). Moreover, the co-infection of *S. aureus* and *P. aeruginosa* has also been reported to be more virulent than a single infection. *S. pyogenes* was also highly prevalent in chronic wounds although there was no statistical significance to this.

In our study, *Acinetobacter* spp*.,* which has previously been reported in wounds, was significantly (80%) and exclusively observed in patients with chronic wounds (Gjødsbøl et al., 2006). Moreover, several species were identified, including for the same patient. Of the different species, *A. baumannii* was the most prominent causing wide range of human infections (Rhoads et al., 2012). Indeed, *A. baumannii* is a well-known nosocomial pathogen with a high potential for antimicrobial resistance (Eveillard et al., 2013). More recently, it has been involved in community-acquired infections and war- and natural disaster-related infections, such as war wounds in troops from Iraq and Afghanistan (Eveillard et al., 2013). The implementation of molecular techniques has greatly improved the identification of *Acinetobacter* species; this may explain the diversity of *Acinetobacter* species identified in wounds (*A. baumannii*, *A. nosocomialis*, *A. guangdongensis*, *A. junii*, and *A. lwoffii*) (Rafei et al., 2014; Al Atrouni et al., 2016). In addition, two different species were also identified, according to the area of the wound sampled (*A. lwoffii* from the edge and *A. junii* from the center). All these species have been described as originating from various environmental sources (Al Atrouni et al., 2016). They have previously been identified in human skin, with the exception of *A. guangdongensis* which was first described in 2014 from an abandoned lead-zinc ore mine (Feng et al., 2014; Al Atrouni et al., 2016).

R. felis was also identified in the wound of one patient. This is the first time that this intracellular bacterium has been identified in Guinea (Conakry). It had already been recovered from several countries in sub-Saharan Africa such as Senegal, Gabon, and Kenya/Tanzania, mainly from the blood of febrile and afebrile patients (albeit more frequently in febrile patients). *R. felis* has also been reported in eschars in Senegal as well as in healthy skin swabs (Mediannikov et al., 2013; Mediannikov et al., 2014). The first transmission route reported for *R. felis* was through cat fleas, *Ctenocephalides felis*. Recently, *R. felis* was detected in mosquitoes. It has been also demonstrated that *Anopheles gambiae* mosquitoes may be a vector of the bacterium (Dieme et al., 2015). Another possibility is that the wound may be contaminated by the *R. felis* from environmental sources as booklice, *Liposcelis bostrychophila*, which is systematically infected by *R. felis*

case, it remains unknown whether *R. felis* from *L. bostrychophila* identified in wounds is merely a contamination by insect parts or whether, once inoculated, it plays a role in the infectious process in the wound. Simultaneously, other pathogens which were tested, including *Mycobacterium* spp., *S. pneumoniae*, *Salmonella* spp., *H. ducreyi*, *Leishmania* spp., *Mansonella* spp., and poxvirus, were not detected.

Overall, patients with chronic wounds present a mixed polymicrobial flora compared to the skin of healthy people. However, some results are not always as easy to explain. For example, Crisp *et al.* recently showed that the bacterial cause of cellulitis cannot be determined by comparing the prevalence and quantity of pathogens from infected and uninfected skin biopsy specimens using current molecular techniques (qPCR and pyrosequencing) as well as standard culture techniques (Crisp et al., 2015).

CONCLUSIONS

The clinical management of chronic wounds is a real challenge, particularly in patients with comorbidity and who live in poor areas. Our findings confirm that chronic wounds are colonized by multiple bacterial species as several bacterial species were observed in the skin, mainly from chronic wounds, in Guinea (Conakry). *P. aeruginosa* and *S. aureus* were the more prevalent species identified. Several different species of *Acinetobacter* were also detected, including one, *A. guangdongensis*, which was identified for the first time in skin. *R. felis* was also observed for the first time in this country. Chronic wounds are colonized by many different bacteria which can promote wound enlargement or delay healing.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of Guinea, Conakry (agreement number 008/CNERS/14). Written informed consent from all participants, including patients and the parents or legal guardians of children was obtained.

Consent for publication

All authors have approved the manuscript for submission.

Availability of data and materials

All relevant data are within the paper.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by Méditerranée Infection and the National Research Agency under the program « Investissements d'avenir », reference ANR-10-IAHU-03.

Authors' contributions

AKK, OM, and FF conceived and designed the framework of this paper. CBE, AKK, AHB, AC, NA, JDN, and OM performed the experiments. CBE, AKK, DR, OM, and FF analyzed the data. CBE, AKK, AHB, AC, and JDN contributed reagents, materials and analysis tools. CBE, DR, OM, and FF wrote the paper. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

We are grateful to the infected and healthy volunteers who agreed to participate in the study, as well as to Mamadou Saliou Toure, Daouda Camara and the staff of Primary Health Care center of Maferinyah for technical assistance.

REFERENCES

Al Atrouni A, Joly-Guillou ML, Hamze M, Kempf M (2016). Reservoirs of Non-*Acinetobacter baumannii* Species. Front Microbiol.7:49.

Angelakis E, Mediannikov O, Parola P, Raoult D (2016). *Rickettsia felis*: The complex journey of an emergent human pathogen. Trends Parasitol. 32:554-64.

Benotmane A, Mohammedi F, Ayad F, Kadi K, Azzouz A (2000). Foot lesions: etiologic and prognostic factors. Diabetes Metab. 26:113-117.

Bessa LJ, Fazii P, Di Giulio M, Cellini L (2015). Bacterial isolates from infected wounds and their antibiotic susceptibility pattern: some remarks about wound infection.

Bouvresse S, Socolovshi C, Berdjane Z, Durand R, Izri A, Raoult D, Chosidowa O, Brouqui P (2011). No evidence of *Bartonella quintana* but detection of *Acinetobacter baumannii* in head lice from elementary school children in Paris. Comp Immunol Microbiol Infect Dis.34:475-7.

Crisp JG, Takhar SS, Moran GJ, Krishnadasan A, Dowd SE, Finegold SM, H. Summanen H, Talan DA (2015). Inability of polymerase chain reaction, pyrosequencing, and culture of infected and uninfected site skin biopsy specimens to identify the cause of cellulitis. Clin Infect Dis.61:1679-87.

Dieme C, Bechah Y, Socolovschi C, Audoly G, Berenger JM, Faye O (2015). Transmission potential of *Rickettsia felis* infection by *Anopheles gambiae* mosquitoes. Proc Natl Acad Sci U S A.112:8088-93.

Dowd SE, Wolcott RD, Sun Y, McKeehan T, Smith E, Rhoads D (2008). Polymicrobial nature of chronic diabetic foot ulcer biofilm infections determined using bacterial tag encoded FLX amplicon pyrosequencing (bTEFAP). PLoS One.3:e3326.

Ehounoud CB, Yao KP, Dahmani M, Achi YL, Amanzougaghene N, Kacou N'D, N'Guessan JD, Raoult D, Fenollar F, Mediannikov O (2016). Multiple Pathogens Including Potential New Species in Tick Vectors in Côte d'Ivoire. PLoS Negl Trop Dis.10:e0004367.

Essayagh M, Essayagh T, Essayagh S, El Hamzaoui S (2014). Epidemiology of burn wound infection in Rabat, Morocco: Three-year review. Med Sante Trop.24:157-164.

Eveillard M1, Kempf M, Belmonte O, Pailhoriès H, Joly-Guillou ML (2013). Reservoirs of *Acinetobacter baumannii* outside the hospital and potential involvement in emerging human community-acquired infections. Int J Infect Dis.17:e802-5.

Feng GD, Yang SZ, Wang YH, Deng MR, Zhu HH (2014). *Acinetobacter guangdongensis* sp. nov., isolated from abandoned lead-zinc ore. Int J Syst Evol Microbiol.64:3417-21.

Ferdouse F, Hossain MA, Paul SK, Ahmed S, Mahmud MC, Ahmed R, Mahmud MdC, R. Ahmed, Haque AKMF, Nur-a-Alam Khan M, Ghosh S, Urushibara N, Kobayashi N (2015). *Rickettsia felis* Infection among Humans, Bangladesh, 2012-2013. Emerg Infect Dis. 21:1483-5.

Gjødsbøl K, Christensen JJ, Karlsmark T, Jørgensen B, Klein BM, Krogfelt KA (2006). Multiple bacterial species reside in chronic wounds: a longitudinal study, Int Wound J. 3:225-31.

Gomez R, Murray CK, Hospenthal DR, Cancio LC, Renz EM, Holcomb JB, Wade CE, Wolf SE (2008). Causes of mortality by autopsy findings of combat casualties and civilian patients admitted to a burn unit. J Am Coll Surg. 208:348-54.

Gottrup F (2003). Organization of wound healing services: the Danish experience and the importance of surgery. Wound Repair Regen. 11: 452-457.

Guitard J, Edouard S, Lepidi H, Segonds C, Grare M, Ranty-Quintyn ML, Rouquette L, Cointault O, Rostaing L, Kamar N, Fenollar F (2012). Identification of cause of posttransplant cachexia by PCR. Emerg Infect Dis.18:1386-8.

Gulam-Abbas Z, Lutale JK, Morbach S, Archibald LK (2002). Clinical outcome of diabetes patients hospitalized with foot ulcers, Dar es Salaam, Tanzania. Diabet Med. 19:575-579.

Hamad I, Forestier CL, Peeters M, Delaporte E, Raoult D, Bittar F (2015). Wild gorillas as a potential reservoir of *Leishmania* major. J Infect Dis.211:267-73.

Int Wound J. 12:47-52.

Keita AK, Dubot-Pérès A, Phommasone K, Sibounheuang B, Vongsouvath M, Mayxay M, Raoult D, Newton PN, Fenollar F (2015). High prevalence of *Tropheryma whipplei* in Lao kindergarten children. PLoS Negl Trop Dis.9:e0003538.

La Scola B, Gundi VA, Khamis A, Raoult D (2006). Sequencing of the *rpoB* gene and Flanking spacers for molecular identification of *Acinetobacter* species. J Clin Microbiol. 44:827-32.

Lavender CJ, Globan M, Johnson PD, Charles PG, Jenkin GA, Ghosh N, Clark BM, Martinello M, Fyfe JA (2012). Buruli ulcer disease in travelers and differentiation of *Mycobacterium ulcerans* strains from Northern Australia. J Clin Microbiol. 50:3717-21.

Leslie DE, Azzato F, Karapanagiotidis T, Leydon J, Fyfe J (2007). Development of a real-time PCR assay to detect *Treponema pallidum* in clinical specimens and assessment of the assay's performance by comparison with serological testing. J Clin Microbiol. 45:93-6.

Mediannikov O, Fenollar F, Socolovschi C, Diatta G, Bassene H, Molez JF, Sokhna C, Trape JF, Raoult D (2010). *Coxiella burnetii* in humans and ticks in rural Senegal. PLoS Negl Trop Dis.4:e654.

Mediannikov O, Socolovschi C, Edouard S, Fenollar F, Mouffok N, Bassene H, Diatta G, Tall A, Niangaly H, Doumbo O, Lekana-Douki JB, Znazen A, Sarih M, Ratmanov P, Richet H, Ndiath MO, Sokhna C, Parola P, Raoult D (2013). Common epidemiology of *Rickettsia felis* infection and malaria, Africa. Emerg Infect Dis. 19:1775-83.

Mediannikov O, Socolovschi C, Million M, Sokhna C, Bassene H, Diatta G, Fenollar F, Raoult D (2014). Molecular identification of pathogenic bacteria in eschars from acute febrile patients, Senegal. Am J Trop Med Hyg. 91:1015-1019.

Mourembou G, Fenollar F, Lekana-Douki JB, Ndjoyi Mbiguino A, Maghendji Nzondo S, Matsiegui PB, Manego RZ, Ehounoud CH, Bittar F, Raoult D, Mediannikov O (2015b). *Mansonella*, including a Potential New Species, as Common Parasites in Children in Gabon. PLoS Negl Trop Dis.9:e0004155.

Mourembou G, Lekana-Douki JB, Mediannikov O, Nzondo SM, Kouna LC, Essone JC, Biteghe Bi, Fenollar F, Raoult D (2015a). Possible Role of *Rickettsia felis* in Acute Febrile Illness among Children in Gabon. Emerg Infect Dis. 21:1808-15.

Mourembou G, Nzondo SM, Ndjoyi-Mbiguino A, Lekana-Douki JB, Kouna LC, Matsiegui PB, Manego RZ, Moukandja IP, Keïta AK, Tissot-Dupont H, F. Fenollar F, Raoult D (2016). Cocirculation of *Plasmodium* and Bacterial Deoxyribonucleic Acids in Blood of Febrile and Afebrile Children from Urban and Rural Areas in Gabon, Am J Trop Med Hyg. 95:123-32.

Parola P, Mediannikov O, Dieme C, Raoult D (2015). Reply to Slesak, et al: So much about *Rickettsia felis* infection to be discovered. Proc Natl Acad Sci U S A. 112:6595-6.

Percival SL, Thomas JG, Williams DW (2010). Biofilms and bacterial imbalances in chronic wounds: anti-Koch. Int Wound J.7:169-75.

Pratt B, Cheesman J, Breslin C, Do MT (2016). Occupational injuries in Canadian youth: an analysis of 22 years of surveillance data collected from the Canadian Hospitals Injury Reporting and Prevention Program. Health Promot Chronic Dis Prev Can. 36:89-98.

Rafei R, Kempf M, Eveillard M, Dabboussi F, Hamze M, Joly-Guillou ML (2014). Current molecular methods in epidemiological typing of *Acinetobacter baumannii*. Future Microbiol. 9:1179-94.

Rhoads DD, Wolcott RD, Sun Y, Dowd SE (2012). Comparison of culture and molecular Identification of bacteria in chronic wounds. Int J Mol Sci. 13: 2535-2550.

Rolain JM, Raoult D (2005). Molecular detection of *Coxiella burnetii* in blood and sera during Q fever. QJM. 98:615-7; author reply 617-20.

Rolain JM, Sthul L, Maurin M, Raoult D (2002). Evaluation of antibiotic susceptibilities of three rickettsial species including *Rickettsia felis* by a quantitative PCR DNA assay. Antimicrob Agents Chemother.46: 2747– 2751.

Sano D, Tieno H, Drabo Y, Sanou A (1998). Management of the diabetic foot, apropos of 42 cases at the Ougadougou University Hospital Center. Dakar Med. 43:10-113.

Scales BS, Huffnagle GB (2013).The microbiome in wound repair and tissue fibrosis. J Pathol. 229:323-31.

Sen CK, Gordillo GM, Roy S, Kirsner R, Lambert L, Hunt TK, Gottrup F, Gurtner GC, Longaker MT (2009). Human skin wounds: a major and snowballing threat to public health and the economy. Wound Repair Regen. 17:763-71.

Serra R, Grande R, Butrico L, Rossi A, Settimio UF, Caroleo B, Amato B, Gallelli L, de Franciscis S (2015). Chronic wound infections: the role of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Expert Rev Anti Infect Ther.13:605-13.

Smith A (2004). Etiology of the Problem Wound. In: Sheffield PJ, Fife CE, Smith APS, eds. Wound Care Practice, Flagstaff: Best Publishing Company. 3-48.

Socolovschi C, Mediannikov O, Sokhna C, Tall A, Diatta G, H. Bassene H, Trape JF, Raoult D (2010). *Rickettsia felis*, a common cause of uneruptive fever in rural Senegal. Emerg Infect Dis. 16:1140-2.

Thomsen TR, Aasholm MS, Rudkjobing VB, Saunders AM, Bjarnsholt T, Givskov M, Kirketerp-Møller MK, Nielsen PH (2010). The bacteriology of chronic venous leg ulcer examined by culture-independent molecular methods. Wound Repair Regen.18:38-49.

Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr (2015). *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev.28:603-61.

Wansbrough-Jones M, Phillips R (2006). Buruli ulcer: emerging from obscurity. Lancet. 367:1849-58.

Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, Nouwen JL (2005). The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis. 5:751-62.

Wolcott RD, Cox SB, Dowd SE (2010b). Healing and healing rates of chronic wounds in the age of molecular pathogen diagnostics. J Wound Care. 19:272-8, 280-1.

Wolcott RD, Rhoads DD, Bennett ME, Wolcott BM, Gogokhia L, Costerton JW, Dowd SE (2010a). Chronic wounds and the medical biofilm paradigm. J Wound Care. J Wound Care. 19:45-6, 48-50, 52-3.