Bacteriological Profile and Antibiotic Resistance Patterns of Pus/Wound Samples in Humans with Infected Wounds in North Central Algeria

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Abstract

The present study was carried out between 2016 and 2020 in Makour Hamou Public Hospital, Ain Defla district, North Centre of Algeria. The study aimed to characterise the antimicrobial resistance and multidrug resistance in bacteria isolated from 620 patients with purulent skin wounds. Out of the 428 bacterial isolates, 283 were Gram-positive (66.12 ± 4.48%) (P<0.001). A total number of 77 Staphylococcus aureus isolates were obtained, among them 31.2 ± 9.3% (24/77) were methicillin-resistant. The most frequent Gram-negative bacteria were Escherichia coli (30.34±7.4%), followed by Klebsiella pneumoniae (25.52±7.10%), and Pseudomonas aeruginosa (23.45±6.70%). All Staphylococcus aureus isolates (77/77) were sensitive to clindamycin. Escherichia coli isolates were resistant to several antibiotics with high resistance rates to amoxicillin (38/44; 86.4 ± 10.1%), amoxicillin-clavulanate (30/44; 68.2 ± 13.8%), cefazolin (21/44; 47.7 ± 7.5%) and trimethoprim-sulfamethoxazole (16/44; 36.4 ± 14.2%) (P<0.001). All Gram-negative bacteria were sensitive to amikacin (145/145) and only one Gram-positive isolate (99.65 ± 0.69%) was resistant to vancomycin. Multidrug resistance was observed in 31.54% of isolates; it was significantly higher in Gram-negative compared to Gram-positive bacteria (62/145; 42.76 ± 8% and 73/283; 25.79 ± 5.10%, respectively) (P<0.001). Multidrug resistance rate was significantly correlated to patients’ age (P<0.001) but not according to years. These results showed the presence of different bacteria species from human wound infections. The resistance to one or multiple antibiotics were frequent. It is recommended to reduce irrational use of antibiotics and a more frequent use of antibiogram before any antibiotic prescription.

Keywords: Multidrug Resistance, Antibiotic Susceptibility, Pus, Wound, Algeria
INTRODUCTION

Antibiotic resistance is a critical cosmopolitan public health issue causing increasing serious public concerns. The emergence of multidrug resistant bacteria is increasingly common and represents a threat in all over the world.\(^1\)\(^-\)\(^3\) This bacterial resistance is the main cause of a multitude of difficult-to-treat wound infections.\(^4\) Several epidemiological studies showed important fluctuations in antibiotic resistance according to the nature of wounds, surgical procedures, and regions.\(^5\) Different patterns of bacteria such as staphylococci, Enterobacteriaceae, and Pseudomonas, cause of pus infections.

Depending on the causative bacteria, active infections are either purulent or serosanguinous.\(^6\) These pyogenic infections interfere with the wound healing process, leading to a dramatic increase of treatment costs, morbidity, and mortality.\(^7\)\(^,\)\(^8\) Bacteria identification, to the level of species and strain, and monitoring of the organisms’ response to the therapeutic protocol require microbiological research implementation in both surgery and pharmacology.\(^6\) Knowing the local epidemiology and the antibiotic resistance pattern will therefore help decreasing treatment failure risks.\(^7\) The intensive uncontrolled use of antibiotics increases microbial resistance rate and speed. This resistance induces the development of chronic wounds and in extreme cases stops the healing.\(^8\)

In the world, several research were conducted to estimate the frequency of pyogenic infections, causative bacteria, and their antibiotic susceptibility. Different wound infection rates were reported in several countries. It was reported to be low in France (2.2%)\(^10\) but very high in developing countries such as Sudan (25.23%).\(^11\)

In Algeria, the incidence of methicillin-resistant \textit{S. aureus} (MRSA) isolated from surgical site infections between 2008 and 2010 was estimated to 4.2%.\(^12\) In the north west of Algeria, Bouharkat et al reported that 18.3% of the 60 \textit{S. aureus} isolates were MRSA.\(^13\) However, there is lack of information regarding bacterial resistance evolution in Algeria.

The present study aimed to investigate antimicrobial resistance patterns, including multidrug resistance, among bacteria isolated from various types of pyogenic infected wounds in humans in Makour Hamou Hospital in Ain Defla, Algeria.

MATERIALS AND METHODS

Samples’ collection

The present study was a prospective investigation. It was carried out during five years, from the 1st of January 2016 to the 31st of December 2020 in Makour Hamou public hospital, Ain Defla district, North Centre of Algeria. A total of 620 patients consisting of 262 males and 358 females (sex ratio M:F = 0.73) presenting wound purulent infections and at least one of the wound infection symptoms were including in the sampling.\(^14\) The patients’ ages ranged from one month to 90 years (mean age: 37.7±20.69 years).

Pus/wound samples were collected aseptically using disposable sterile swabs or syringes. The samples were identified and immediately transported to the laboratory of medical analyses. To prevent duplicate samples, each patient was included in the study only once during their entire hospitalization period.

Bacterial isolation and identification

The samples were primarily cultivated using standard media: blood agar, Mannitol salt agar, and Heketone agar (Tmmedia Labs, Rajasthan, India) and incubated at 37°C for 24-48 h. Blood agar plates were incubated in an atmosphere rich in CO\(_2\). In the case of a polymicrobial culture, distinct colonies were randomly selected and streaked into new plates until pure cultures were obtained. The identification was made according to standard bacteriological diagnostic procedures: macroscopic examination of colonies, unstained wet-mount test, Gram staining, and biochemical tests. Control strains as \textit{E. coli} (ATCC 25922), \textit{S. aureus} (ATCC 25923), and \textit{P. aeruginosa} (ATCC 27853), were included in the study.

Antimicrobial susceptibility testing

Antibiotic susceptibility test was performed using Muller-Hinton agar (Tmmedia Labs, Rajasthan, India) plates by disk diffusion method according to Guidelines of Clinical and Laboratory Standards Institute.\(^15\) A total number of
26 antibiotic discs (Cypress Diagnostic, Langdrop, Belgium) were used to test antibio-susceptibility of the strains (Table 1).

Methicillin-resistant <i>S. aureus</i> was detected by disc diffusion test using cefoxitin (30 µg) on Muller-Hinton Agar. Bacteria producing Extended-Spectrum β-lactamases (ESBLs) were screened using disc diffusion on Muller-Hinton Agar and confirmed by double disc method (Amoxicillin-Clavulanate (20/10 µg)/Cefotaxime (30 µg)).

Data analysis
All data were analysed in a Microsoft Excel® 2019 spreadsheet. Percentages were compared using chi-square test at 5% threshold with Epi info 2000 software.
RESULTS

Description of the study population

Between 2016 and 2020, 620 pus samples were collected from different types of infected wounds where the highest number of samples was collected during 2017 (160/620; 25.81 ± 3.44%). More than half (373/620; 60.16 ± 3.85%) of the infected wounds were collected between October and April, corresponding to the cold months ($P<0.001$) (Table 2). The highest number of samples was collected from patients aged between 30 and 35 years (76/620; 12.26 ± 2.58%), followed by [35-40] age group (66/620; 10.65 ± 2.43%) and finally [25-30] age group (54/620; 8.71 ± 2.22%) (Figure 1).

Bacterial species

Among the 620 cultivated swab samples, 407 were positive (65.65 ± 3.74%) totalling 428 bacterial isolates. Among the positive samples, 21 showed mixed cultures (5.16 ± 4.86%). Gram-positive bacteria (283; 66.12 ± 4.48%) were significantly more frequent than Gram-negative bacteria (145; 33.88 ± 4.48%) ($P<0.001$). The majority of Gram-negative bacilli were *Escherichia coli* (*E. coli*) (44/145; 30.34 ± 7.48%) followed by *Klebsiella pneumoniae* (*K. pneumoniae*) (37/145; 25.52 ± 7.10%) and *P. aeruginosa* (34/145; 23.45 ± 6.70%) ($P<0.001$). Coagulase-negative staphylococci (CoNS) were the most frequent (154/283; 54.42 ± 5.80%) Gram-positive cocci, followed by *S. aureus* (77/283; 27.21

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<th>(% ± SE)</th>
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Figure 1. Number of pus samples collected in the present study according to age category

Table 2. Characteristics of the studied human population in the present study
### Table 3. Antibiotic resistance pattern of bacterial strains isolated in the present study

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<tr>
<th>Bacterial isolate</th>
<th>CoNS (N=154)</th>
<th>S. aureus spp. (N=49)</th>
<th>Streptococcus spp. (N=3)</th>
<th>Enterococcus spp. (N=44)</th>
<th>E. coli (N=44)</th>
<th>Klebsiella pneumoniae (N=37)</th>
<th>P. aeruginosa (N=34)</th>
<th>P. fluorescens (N=4)</th>
<th>Proteus vulgaris (N=10)</th>
<th>Proteus mirabilis (N=9)</th>
<th>Other Enterobacteriaceae (N=7)</th>
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Antibiotics tested: PEN (PEN), GEN (GEN), AMK (AMK), ERY (ERY), CLI (CLI), VAN (VAN), OFX (OFX), SXT (SXT), DOX (DOX), Rif (Rif), Amx (Amx), Amc (Amc), CFZ (CFZ), CTX (CTX), IPM (IPM), FOF (FOF), CIP (CIP)
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<th>Bacterial isolate (number)</th>
<th>CoNS (N=154)</th>
<th>S. aureus (N=77)</th>
<th>Streptococcus spp. (N=49)</th>
<th>Enterococcus spp. (N=3)</th>
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<td>6/37 (16.2 ± 11.9)</td>
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<td>11/44 (25 ± 12.80)</td>
<td>16/37 (43.24 ± 15.96)</td>
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<td>31/77 (40.26 ± 10.96)</td>
<td>8/49 (16.3 ± 10.3)</td>
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<td>22/44 (50 ± 14.8)</td>
<td>20/37 (54.1 ± 16.1)</td>
<td>3/34 (8.8 ± 9.5)</td>
<td>0/4</td>
<td>7/10</td>
<td>4/9</td>
<td>6/7</td>
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</table>

Penicillin (PEN); Gentamicin (GEN); Amikacin (AMK); Erythromycin (ERY); Clindamycin (CLM); Vancomycin (VAN); Ofloxacin (OFLX); Trimethoprim+ Sulfamethoxazole (SXT); Doxycycline (DOK); Rifampicin (RIF); Amoxicillin (AMX); Amoxicillin Clavulanate (AMC); Cefazolin (CFZ); Cefotaxime (CTX); Imipenem (IPM); Fosfomycin (FOF); Ciprofloxacin (CIP); Nalidixic Acid (NAL); Nitrofurantoin (NFT); Ticarcillin (TIC); Ticarcillin-Clavulanate (TIM); Piperacillin (PIP); Ceftazidime (CAZ); Netilmicin (NET); Tobramycin (TOB); Aztreonam (ATM); MDR (Multidrug resistance).

(-): Non-tested
± 11.21%) and *Streptococcus* spp. (49/283; 17.31 ± 4.41%) (*P*<0.001) (Figure 2).

**Antimicrobial resistance patterns**

Among the 77 *S. aureus* isolates, the highest rate of resistance was for penicillin (54/77; 70.1 ± 10.2%), followed by doxycycline (21/77; 27.3 ± 10%), and gentamicin (16/77; 20.8 ± 9.1%) (*P*<0.001). High susceptibility rates of 91.7% and 100% were observed for clindamycin and vancomycin, respectively. Finally, almost one third (24/77; 31.2 ± 9.3%) of *S. aureus* isolates were MRSA. CoNS isolates were resistant to penicillin (135/154; 53.60 ± 7.88%), erythromycin (65/154; 32.47 ± 7.40%), and doxycycline (62/154; 26.62 ± 6.98%) (*P*<0.001). Ticarcillin (19/49; 38.8 ± 13.6%), clindamycin (15/49; 30.6 ± 12.9%), and erythromycin (14/49; 28.6 ± 12.6%) had the highest resistance rates in *Streptococcus* spp. isolates (*P*<0.001).

*Escherichia coli* isolates were resistant to several antibiotics, particularly amoxicillin (38/44; 86.4 ± 10.1%), amoxicillin-clavulanate (30/44; 68.2 ± 13.8%), cefazolin (21/44; 47.7 ± 7.5%) and trimethoprim-sulfamethoxazole (16/44; 36.4 ± 14.2%) (*P*<0.001).

*Klebsiella pneumoniae* showed high resistance rates to amoxicillin (36/37; 97.3 ± 5.2%), amoxicillin-clavulanate (31/37; 83.8 ± 11.9%), cefazolin (25/37; 67.6 ± 15.1%), and cefixime (18/37; 48.7 ± 16.1%) (*P*<0.001). *P. aeruginosa* isolates were most resistant to piperacillin (13/34; 38.2 ± 16.3%), ticarcillin (10/34; 29.4 ± 15.3%), ticarcillin-clavulanate (6/34; 17.7 ± 12.8%), and netilmicin (5/34; 14.7 ± 11.9%) (*P*<0.001).

Four isolates were ESBL positive, consisting of three (3/37; 8.1%) *K. pneumoniae* and one (1/44; 2.3%) *E. coli* isolates. All Gram-negative bacteria were sensitive to amikacin (145/145) followed by imipenem (98.62 ± 1.9%; 143/145). Vancomycin is the most effective antibiotic against Gram-positive bacteria showing 99.65+/-0.69% (282/283 strains) of susceptibility rate (Table 3).

Penicillins antibiotics had the highest rate of resistance followed by cephalosporins among

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**Figure 2.** Frequency of bacteria isolated in the present study from pus/wound samples
### Figure 3. Heat of the antibiotic resistance profile
Gram-negative bacteria, as indicated by dark squares (Figure 3). Moreover, the lowest antibiotic resistance was due to aminoglycoside antibiotics.

**Multidrug resistance profile**

Almost one third of all isolated bacterial strains (31.54 ± 4.40%; 135/428) were multidrug resistant. The MDR rate was correlated to patients’ age (P<0.001). The rate of MDR was higher in Gram-negative (42.76 ± 8%; 62/145) compared to Gram-positive bacteria (25.79 ± 5.10%; 73/283) (P<0.001). In addition, the number of MDR isolates was higher in Enterobacteriaceae (MDR > 44%) compared to *Pseudomonas* spp. (MDR < 9%) (P=0.004). The MDR rate did not vary with the sampling years (P=0.42).

**DISCUSSION**

The antibiotic resistance limits the choice of antibiotics to treat wound infections. The frequency of these infections varied according to time and region.

The present survey was performed in an Algerian hospital located in Ain Defla district; it allowed the collection of 620 pus samples from infected wounds during five years. The number of wound infections was higher during the cold months (373/620; 60.16%) even though several studies found that infection risks increases during the warm weather. In the United States, there were 26.5% more infection cases in August than in January-20. This discrepancy could be related to the scheduling of surgeries outside the summer season in Algeria where the temperature exceeds 45°C during the summer season. Vickers et al. did not find any statistically significant relation between the climate and frequency of infections in two regions (tropical and subtropical Australian hospitals).

The bacteriological profile of pus/wound swabs revealed that more than half of pus samples showed positive cultures (407/620; 65.65%). The rates obtained here are similar to those reported by Rai et al., who worked on children in Nepal (265/450; 59%) and lower than those reported by Thakal-Mesbah et al., in Algeria (79.12%; 1585 samples), Shimekaw et al. in Ethiopia (72.6%; 201 samples), and Kassam et al. in Tanzania (91.4%; 93 samples) using standard bacteriological diagnostic procedures. Despite the presence of pus and signs of wound infections, the rate of negative cultures was 34.35% (213/620). This could be due to pre-surgical antibio-prevention or early antibiotic prescription during the initial stages of infection. In addition -in these cases- bacteriological identification and antibiotic susceptibility testing are performed after therapeutic failure.

The frequency of Gram-positive (66.12%) was higher compared to Gram-negative bacteria (33.8%). This is consistent with the study of Duvadi et al. in Nepalese Tertiary Care Cancer Patients, in which the rate of Gram-positive isolates (63.9%) outnumbered Gram-negative isolates (36.1%). Similar results were reported also in Nepal by Upreti et al. in 182 samples from patients with clinical features of wound infection. A study performed in Algeria on patients with burn wound infection revealed that 68.95% of swabs contained Gram-negative bacteria (68.95%).

The lower isolation frequency of Gram-negative bacteria can be attributed to their limited ability to survive in the dry conditions of normal skin, allowing the domination of Gram-positive bacteria.

*Escherichia coli* (30.34%), *K. pneumoniae* (25.52%), and *P. aeruginosa* (23.45%) were the major bacteria among Gram-negative isolates. The same trend was reported in several regions in the world. In India, Rai et al. found that *P. aeruginosa* was the most common isolate (45/102; 44%) followed by *K. pneumoniae* (28/102; 27%) and *E. coli* (13/102; 13%) in wound infections among child. In a study performed in Tanzania by Manyahi et al., *P. aeruginosa* was the most frequent Gram-negative bacteria (24/147; 16.3%). Causative bacterial species varies according to geographic area and the wounds’ nature. In Algeria, Tchakal-Mesbah et al. reported that *P. aeruginosa* was the most frequent species (33.91%) followed by *K. pneumoniae* (25.14%) and *Acinetobacter baumannii* (16.37%).

*S. aureus* represented the second most frequent (27.21%) Gram-positive bacteria after CoNS. The same trend was reported in 8,569 French patients (35% and 23% for CoNS and *S. aureus*, respectively). Staphylococci are the most common colonizers bacteria of the skin and the
In the present study, we get favourable clinical responses to treatments with vancomycin against Gram-positive infection. In Ethiopia, Mama et al. reported that all the 145 isolates were sensitive to vancomycin. This is a glycopeptide antibiotic used as last-resort treatment against resistant infections. Several generations of vancomycin were developed to prevent conceivable resistance, they are only used in hospitals and is administered intravenously.

Amikacin and imipenem were the effective against Gram-negative bacteria. Both of them are expensive, they have only clinical use. Amikacin is characterized by a high level of resistance to bacterial enzyme modifications; as a result, several Gram-negative bacteria are susceptible to this antibiotic.

The high rate of antibiotic resistance to penicillins is due to the misuse of these antibiotics. Also, the important level of resistance to the cephalosporins antibiotic can be related to their frequent used in surgery. To limit the emergence and spread of resistance to aminoglycosides, it is imperative to minimize selective pressures that arise within hospital settings and areas with unregulated antibiotic usage. Continuous monitoring of resistance genes in humans, animals, and food sources is vital to delay the dissemination of aminoglycoside resistance.

In the present study, 31.54% of isolated strains showed a MDR. This could delay the wound healing process and even alter the patient’s general condition causing for example septicaemia. Furthermore, this resistance reduces the therapeutic choice and increases duration of hospitalizations. In Ethiopian investigations, higher proportions of MDR were recorded from different wound types that were estimated between 80.8 and 85%. In the present study, the MDR in Gram-negative isolates represented 42.76% while 25.79% of Gram-positive bacteria were MDR. These resistance levels were lower than the findings of Duwadi et al. (60 and 40% for Gram-positive and Gram-negative bacteria, respectively). In addition, the MDR varied significantly with bacterial species. There was a significant correlation between age and MDR rate. This could be explained by the build-up with age, the irrational use of antibiotics, especially self-medication.

CONCLUSION

In this study, CoNS and S. aureus are the predominant bacteria isolated from pus/ wound infections from patients at Ain Defla district, North Centre Algeria. Very high resistance rate has been recorded to commonly used antibiotics like amoxicillin (more than 80% for the different bacterial species). Amikacin and vancomycin

primary causes of nosocomial infections and skin infections in the community.

Among S. aureus isolates, 31.17% (24/77) were MRSA. Zerouki et al. found that S. aureus was isolated in 39.5% (30/76) of the Department of Orthopaedic Surgery and Traumatology, University Military Hospital of Constantine, Algeria, among them, 63.3% was MRSA. Also, in Algeria, Bouharkat et al. revealed that S. aureus is the most isolated species (25.97%) in diabetic patients suffering of foot infections where MRSA represented 18.3%. In Morocco, S. aureus rate in burn wound infections was 33.85% (44/86), MRSA represented 86.36% of them. These discrepancy between studies can be explained mainly by the microbial ecology of the hospitals. The high prevalence of S. aureus is expected since this species belongs to the normal flora of healthy persons’ skins.

In the present study, the rate of penicillin resistance was 70.13% for S. aureus. In Morocco, the highest rate was noted for S. aureus (86.36%). According to Que et al., penicillinase production is the most common mechanism liable for the emergence of penicillin resistance where the prevalence is close to 80% between hospitals and the community.

A high level of amoxicillin resistance was found in E. coli (86.36%), K. pneumoniae (97.30%), and other Enterobacteriaceae species. In addition, the combination amoxicillin-clavulanate has very important resistance (68.18 and 83.78% for E. coli and K. pneumoniae, respectively). Furthermore, El Hamzaoui et al. found that more than 80% of the different strains were resistant to this antibiotics association. The most common reason for resistance to β-lactam antibiotics among Enterobacteriaceae is the expression of β-lactamase enzymes that hydrolyse the β-lactam ring, resulting in antibiotic inactivation.

In the present study, the rate of penicillin resistance in E. coli, K. pneumoniae, and other Enterobacteriaceae species was 86.36%. In addition, the combination amoxicillin-clavulanate has very important resistance (68.18 and 83.78% for E. coli and K. pneumoniae, respectively). Furthermore, El Hamzaoui et al. found that more than 80% of the different strains were resistant to this antibiotics association. The most common reason for resistance to β-lactam antibiotics among Enterobacteriaceae is the expression of β-lactamase enzymes that hydrolyse the β-lactam ring, resulting in antibiotic inactivation.
were the most effective against Gram-negative and positive bacteria, respectively. Almost one-third of the isolates were multidrug resistant. This resistance was higher among Gram-positive bacteria. To slowdown the bacterial resistance emerging, an antibiotic susceptibility test before any antibiotic therapy is mandatory. Also, the respect of hygienic practices is necessary to prevent infections, particularly nosocomial infections. Furthermore, increasing public awareness of the risks associated with self-medication. Additional epidemiological studies are needed to perform risk analysis of antibiotic resistance in Algerian bacterial strains. Similarly, genetic background investigation is needed to understand the mechanisms of these antibiotic resistance.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

AUTHORS’ CONTRIBUTION
AA and BK conceptualized the study. AA performed laboratory work, software and formal analysis. AA, RK and MA applied methodology. AA wrote the manuscript. RK and BK reviewed the manuscript. RK and BK supervised and administered the project. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY
All datasets generated or analysed during this study are included in the manuscript.

ETHICS STATEMENT
This study was approved by the Institutional Ethics Committee, Algerian legislation and LBRA’s laboratory with reference number 303/PG-ISV/2015.

INFORMED CONSENT
Written informed consent was obtained from the participants before enrolling in the study.

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