Multidrug Resistant Bacterial Profile and Patterns for Pus Isolates and Recurrent Wound Infections in Nongovernmental Hospitals of Jordan

Hashem A. Abu-Harirah¹, Kawther Amawi¹, Ammar S. Ali Deeb¹, Haytham M. Daradka², Nawal Fares¹, Anwar D. Maraqa³, Audai Jamal Al Qudah⁴ and Emad Daabes⁵

¹Faculty of Allied Medical Sciences- Zarqa University, Jordan.
²Department of Medical Laboratory Sciences, Faculty of Pharmacy, Jadara University, Irbid, Jordan.
³Faculty of Allied Medical Sciences, Al-Ahliyya Amman University, Jordan.
⁴Islamic Hospital, Jordan.
⁵Israa Islamic Hospital, Jordan.

Authors’ contributions

This work was carried out in collaboration among all authors. Author HAAH planned the basic framework for manuscript designed the study. Authors KA and ASAD conducted the analysis and improved the final version of manuscript. Author HMD managed the literature searches. Authors NF and ADM performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AJAQ and ED collected the data and prepared the initial manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Many types of infection can cause pus Infections involving the bacteria; E.coli, so the assessment of multidrug Bacterial profile and patterns is needed to understand the source and management of these injuries.

Purpose: To determine infections and patterns toward antibiotics of pus isolates and recurrent wound infections in nongovernmental hospitals of Jordan.

Methods: During period eleven months, 607 Patients were involved, out of which 128 patients had pus samples and/or recurrent wound infections. Data analysis was done using SPSS version 20.
value was set at <0.05.

**Results:** One hundred twenty eight (21.1%) out of 607 patients were identified to had pus isolates and/or wound recurrent infections 86(87%) out of 128 patients had infections with known pathogenic microbes. Microbiological culture pattern was total of 19 different pathogenic microorganisms were isolated from the participants, with mixed gram-positive and gram-negative species; percentage of 37% gram-positive aerobic bacteria and 63% gram-negative aerobic bacteria.

**Conclusion:** The global burden from multidrug resistant bacteria highly impacted in wound and pus-causing infections, either in hospital acquired infections or community acquired infections. The main causative agents of recurrent wound infection were *Staph. aureus* MRSA, *E. coli*, *Pseudomonas aeruginosa*, *Acinetobacter* spp (MDR). Gram-negative bacteria caused the most of infections by more than 67% comparing with gram-positive bacteria.

**Keywords:** Pus and wound infections; antibiotic sensitivity; Jordan.

# 1. INTRODUCTION

Pus is a thick fluid containing dead tissue, cells, and bacteria, as a “waste of wore” when the body fighting off an infection. Pus infections could be caused by viral, fungal, or bacteria enter your body through broken skin, inhaled droplets from a cough or sneeze and/or poor hygiene [1]. In addition, could be happened Post-operative surgical site infections (SSIs) as recurrent infections of the wounds and causing high morbidity and increased medical expense.

The wound viral infections could be caused by: Herpes viruses, such as the Herpes simplex virus types I and II (diseases: orolabial herpes, genital herpes, eczema herpeticum or Papilloma viruses), or fungal like yeast infections with Candida spp., the most common cause is bacterial infections, many types of bacteria can cause pus Infections involving the bacteria; *E. coli*, *Staphylococcus aureus* or, *Streptococcus pyogenes* are especially prone to pus. Both of these bacteria release toxins that damage tissue and create pus and/or anaerobic Clostridium perfringens: myonecrosis, so-called gas gangrene or aerobic like *S. aureus*. Abscess in general is formed by pus due to breaking down of tissue [2,3]. Abscesses can form on the skin’s surface or inside your body. Wound diseases represent 70-80% death rate. Despite their starting point, all injuries may transmitted by microorganisms or outside bodies or both to another area. Anaerobic microorganisms forms, 33% of the accumulated number of microbial species in colonized injuries, and their number increments increases to around half-in contaminated injuries [4-7]. Microbe of *Pseudomonas aeruginosa* could be one of the most causative agent for post-surgery wound infections, also the nosocomial infection which caused by multi-drug resistant, in the United States MRSA infections have been reported among persons without such exposure (community - acquired MRSA in the United States the incidence ranging from 15 to 74 percent and the incidence rate in European countries is below 5%, Gram negative bacteria and methicillin-resistant *Staphylococcus aureus* (MRSA) in addition to *Pseudomonas aeruginosa* are the most common causes and most complicated cases of wound and systemic infections resulting in significant morbidity and mortality rates in burns patients and , listed available treatment options for the infections [3,4,8,9].

Multidrug-resistant (MDR) bacteria are an emerging globally specially with immunocompromised patients especially group of ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*) pathogens particularly the most aggressive agent which is methicillin resistant *Staphylococcus aureus* (MRSA), spreader globally and became one of the most serious contemporary challenges to the treatment of hospital-acquired infections [10]. Many pathogens are reported as causative agents for wound infections, some of these microbes were aggressive and had complicated infections, methicillin resistant *Staphylococcus aureus* (MRSA) is estimated to cause nineteen thousands of deaths per year in the United States, also the vancomycin - resistant S. aureus (VRSA) became a new scourge in hospitals. The multidrug resistant (MDR) and pan drug -resistant (PD) gram-negative bacteria, is less prevalent than MRSA. Recently, the MDR and extensively drug resistant (XDR) strains of *Mycobacterium tuberculosis* were starting to make an actual threat in the developing world [9,11,12-17].
The aim of this study is for assessing of multidrug resistant bacterial profile and patterns for pus isolates and recurrent wound infections in nongovernmental hospitals of Jordan in order to understand the source and management of such infections in nongovernmental hospitals of Jordan.

2. MATERIALS AND METHODS

2.1 Study Area

This was for more than months period from 1-1-2018 to 11-11-2018 for both of in and outpatients in one of the biggest nongovernmental hospitals in Jordan.

In and outpatients were involved from surgery and admitted wards. All the patients fulfilled the criteria above were consecutively enrolled in the study.

2.2 Study Design and Study Population

A hospital base study was performed to determine the multidrug resistant bacterial profile and patterns for pus isolates and recurrent wound infections in nongovernmental hospitals of Jordan and the percent of infections among the causative agents, within the period from 1-1-2018 to 11-11-2018 for both of in and outpatients in one of the biggest nongovernmental hospitals in Jordan.

2.3 Sample Size Determination and Samplings Techniques

The total number of patients for the study was 607; 331 of them wound infected patients, which forms 54.4% of the total population the female number of patients was 124 females (63%) and the male’s numbers was 207 (37%). The pus culture’s patients were 128, which forms 21.1% of the total population, the female number of pus patients was 40 females (31%) while the male’s numbers were 88 (69%). The overall percent female over male 41/59. The aim and benefits of the experiment was clearly illustrated for the participants prior to data collection, the participation was on voluntary basis and they have informed them it is there right to withdraw from the study at any time during the course of data collection.

2.4 Sample Collection and Laboratory Quality Control and Methods

Data were from volunteers collected using structured questionnaire consisting of the patient's demographic information, two sets of deep wound samples were obtained by rolling two sterile swab sticks one after the other over the surface of the sampling site, Biopsies and aspirated material were preferred over swabs for deeper wound Site, after debridement of superficial exudates, one swab specimen was immediately transferred into a thioglycollate medium, and sent with the second specimen to the microbiology laboratory for analysis under the supervision of medical microbiologist. Appropriate conditions had created for aerobic and anaerobic bacteria and fungi to multiply and isolate a pure culture of microorganisms in culture medium to determine and identify the type of organism in wound, pus, soft tissue, diabetic foot, skin, ulcer, cyst, bile, abscess or any sterile swab, in addition to the antibiotic susceptibility testing. Agar’s expiration date and QC, cracked dishes, thin or unequal fill, hemolysis, evidence of freezing, desiccation, bubbles and contaminated agars had excluded, the performance of prepared media was tested by inoculation control stands; S. aureus ATCC-25923 and E. coli ATCC-25922 to confirm the results.

2.5 Antimicrobial Agents and Antibiotics Susceptibility Testing

The isolated organisms were inoculated onto nutrient agar plates, and anti-microbial susceptibility testing was carried out using the modified Kirby-Bauer disc diffusion method, discs for available, anti-microbial agents were used. Attempts were made to incorporate discs representative of different classes of antimicrobials. Disc of the following anti-microbials was used: Ceftriaxone (30 µg), Ceftizoxime (30 µg), Cefoxitin (30 µg), Gentamicin (10 µg), Amoxicillin/Clavulanate (30 µg), Cefuroxime (30 µg), Nitrofurantoin (100 µg), Ceftazidime (30 µg), Ciprofloxacin (10 µg), Ofloxacin (10 µg), Pefloxacin (30 µg), Clindamycin (2 µg), Ampicillin/Sulbactam (10/10 µg), Imipenem (10 µg), Meropenem (10 µg), Ertapenem (10 µg), Clarithromycin (10 µg), Ampicillin (30 µg), Erythromycin (10 µg), Ampicillin/Cloxacillin (30 µg), Cefixime (5 µg), Levofloxacin (10 µg), Norfloxacin (10 µg), And Metronidazole (5 µg).

2.6 Data Analysis and Interpretation

Data analysis was done using Statistical Package for the Social Sciences (SPSS) version 23 (IBM Corp, 2015). Qualitative data were described as proportions or percentages; cross-
tabulation was used where necessary. Test of significance for differences for quantitative and categorical variables was tested with T-test and Chi-square analyses respectively. A p-value of < 0.05 was considered significant.

3. RESULTS

3.1 Sociodemographic Characteristics of Study Participants

General characteristics of study population: the total number of patients for the study was 607; 331 of them wound infected patients, which forms 54.4% of the total population. The female number of patients was 124 females (63%) and the male's numbers was 207 (37%). The pus culture’s patients were 128, which forms 21.1% of the total population. The female number of pus’s patients was 40 females (31%) while the male’s numbers was 88 (69%). The overall presents female over male 41/59. (Table 1 & Table 2).

Out of 607 total samples; 510(84.0%) samples given growth cultures, and 97(16%) given no growth; for wound samples the growth cultures were 282 samples (85.2%) while the non-growths were 49 (14.8%) samples, but in case of diabetic foot ulcers 130 samples given growth (87.8%) and only 18(12.2%) samples were not grouted, for Pus samples 98(76.6%) were ground and 30 (23.4%) samples were not. (Table 3-A, Table 3-B).

3.2 Bacterial Profile among Wound Infection Suspected Patients

In pus, samples 19 pathogens were isolated, 98 types of bacteria were isolated, 12 of them was considered as a nonpathogenic bacterium and 30 samples got no growth. *E. coli* was the most frequent pathogen followed by 21.4% of the total infections then *S. aureus* by percentage of 14% and *S. aureus* (MDR) by percentage of 11% then Nonhemolytic streptococci by percentage of 9%, then the others; *K. pneumoniae*, *Streptococcus agalactiae* (group B), *Morganella morganii*, *E. coli* ESBL, *Streptococcus pyogen*, *K. oxytoca* ESBL, *Acinetobacter* spp (MDR), Vancomycin resistant enterococci, *Pseudomonas aeruginosa*, *P. mirabilis*, *K. pneumoniae* carbapenemase XDR, *E. coli* carbapenemase XDR, Alpha hemolytic streptococci and *Acinetobacter* spp consist about 30% of the total infections (Table 4-A).

| Type of culture                  | Male | Female | Total |
|---------------------------------|------|--------|-------|
| Numbers of patients for Wound swab | 207  | 124    | 331   |
| Percentage of patients for Wound swab | 63%  | 37%    |       |
| Numbers of patients for Diabetic foot swab | 65   | 83     | 148   |
| Percentage of patients for Diabetic foot swab | 44%  | 56%    |       |
| Numbers of patients for Pus for culture | 88   | 40     | 128   |
| Percentage of patients for Pus for culture | 69%  | 31%    |       |
| Total number                     | 360  | 247    | 607   |
| Total percentage                 | 59%  | 41%    | 100%  |

| Types of test          | Number of tests | Percentage of total subjects |
|------------------------|-----------------|------------------------------|
| Diabetic foot swab     | 148             | 24.4%                        |
| Pus for culture        | 128             | 21.1%                        |
| Wound swab             | 331             | 54.5%                        |
| Total                  | 607             | 100.0%                       |

| Types of test                     | Number of tests | Percentage of total subjects |
|-----------------------------------|-----------------|------------------------------|
| Number of growth samples          | 510             | 84.0%                        |
| Number of non growth samples      | 97              | 16.0%                        |
| Total                             | 607             | 100.0%                       |
Table 3B. Growth statistics -number of growth and non-growth sample results of pus samples

| Types of test               | Number of tests | Percentage of total subjects |
|-----------------------------|-----------------|-----------------------------|
| Number of growth samples    | 98              | 76.6%                       |
| Number of non growth samples| 30              | 23.4%                       |
| Total                       | 128             | 100.0%                      |

Table 4-A. Pus samples pathogenic agents

| Type of pathogen                     | Number of infections | Percentage |
|--------------------------------------|----------------------|------------|
| E. coli                              | 21                   | 21.4%      |
| E. coli carbapenemase XDR            | 1                    | 1.0%       |
| E. coli ESBL                         | 4                    | 4.1%       |
| S. aureus                            | 14                   | 14.3%      |
| S. aureus MRSA                       | 11                   | 11.2%      |
| Streptococcus pyogen                 | 3                    | 3.1%       |
| Acinetobacter spp                    | 1                    | 1.0%       |
| Acinetobacter spp(MDR)               | 2                    | 2.0%       |
| Morganella morganii                  | 4                    | 4.1%       |
| K. pneumoniae                        | 5                    | 5.1%       |
| K. pneumoniae carbapenemase XDR      | 1                    | 1.0%       |
| K. oxytoca ESBL                      | 2                    | 2.0%       |
| Streptococcus agalactiae (group B)   | 4                    | 4.1%       |
| Nonhemolytic streptococci            | 9                    | 9.2%       |
| P. mirabilis                         | 1                    | 1.0%       |
| Alpha hemolytic streptococci         | 1                    | 1.0%       |
| Pseudomonas aeruginosa               | 1                    | 1.0%       |
| Vancomycin resistant enterococcus    | 1                    | 1.0%       |
| No pathogenic bacteria               | 12                   | 12.2%      |
| No growth after 48 hours             | 30                   | -          |
| Total number                          | 98                   | 52.0%      |

The High resistant drug isolates pus samples pathogens were; S. aureus MRSA, E. coli ESBL, Acinetobacter spp (MDR), K. oxytoca ESBL, E. coli carbapenemase XDR, Vancomycin resistant enterococcus, K. pneumoniae carbapenemase XDR (see Table 4-B).

Only 14.3% of infections in pus samples caused by multidrug drug resistant bacteria; S. aureus MRSA was the main cause infections by percentage of 11.2% and the second cause was Acinetobacter spp (MDR) by percentage of 3.1% see (Table 4-C).

Table 4-B. High drug resistant and MDR for pus samples

| Type of pathogen                     | Number of infections | Percentage |
|--------------------------------------|----------------------|------------|
| Acinetobacter spp (MDR)              | 2                    | 2.0%       |
| E. coli carbapenemase XDR            | 1                    | 1.0%       |
| E. coli ESBL                         | 4                    | 4.1%       |
| S. aureus MRSA                       | 11                   | 11.2%      |
| Vancomycin resistant enterococcus    | 1                    | 1.0%       |
| K. oxytoca ESBL                      | 2                    | 2.0%       |
| K. pneumoniae carbapenemase XDR      | 1                    | 1.0%       |
| Total number                          | 22                   | 22.4%      |
4. DISCUSSION

Recurrent Wound infections is forming a considered figure of lesions globally. Recently, the world health organization reports in its first ever list of antibiotic-resistant "priority pathogens" a catalog of 12 families of bacteria that pose the greatest threat to human health, in the top of the list the most of the causative pathogens in wound infection is *Staph. aureus* MRSA which is spreading globally to form approximate 20% of wound infections and the prevalence of antibiotic resistant strains of *S. aureus* is increasing on an alarming rate. The highest resistance was recorded against ampicillin and erythromycin (88% each), while resistances against oxacillin, fosfomycin, cefoxitin and ciprofloxacin were also worrisome [18].

As per the results for this study in Jordan, more than 50% of the pus's infections caused by four types of microbes; *Staph. aureus*, *E. coli*, Nonhemolytic streptococci bacteria & *Streptococcus agalactiae* (group B), *Staph. aureus* MRSA infections in pus samples only; 11.2% of total pus-causing infections, while the *Staph. aureus* non-MDR formed 14.3%, the actual hazard of these infections are the resistance against antibiotics either community acquired infections or nosocomial infections in addition to the complications and cost of treatment and managing the infections [5,6,11,16,19].

*Acinetobacter* spp is presented strongly in the results as a causative agents pus-causing microbes which is formed approximate 3.1% of pus's infections in Jordan, 2% for MDR strains and 1% for non MDR species, the risk in *Acinetobacter* spp infections in its membership among ESKAPE group (Enterococcus faecium, *Staphylococcus aureus*, Klebsiella pneumoniae, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, Enterobacter cloacae) and the resistance and spreading within healthcare centers [13,14].

E. coli caused approximate 1/5 over all of the pus's infections by percentage of 21.4%, this showed that the most pus-causing infections caused by *E. coli* non extended spectrum β-lactamase (ESβL), but the percentage of the Nonhemolytic streptococci bacteria was relatively high by percentage of 9.2%, and *Streptococcus agalactiae* (group B) formed 4.1%.

The dread of extended spectrum β-lactamase (ESβL) enzyme to get new ability for developing antimicrobial resistance and multidrug-resistant (MDR) mechanisms, in *Morganella morganii* which was formed about 4.1% of whole wound infections and *Pseudomonas aeruginosa* which is causative agent for post-surgery wound infections and is considered as an important pathogen of both community and hospital acquired infections, and the ability to cause infections as Carbapenemase and MDR agents, also could be important source of concern since these organisms are not only resistant to beta-lactam antibiotics but also show cross-resistance to other groups of antibiotics [8,10, 20] (Table 4-B)& (Table 4-C).

The highly significancy by p<0.001 of MDR results is ringing the bill to focus more in handling and take care for the progression of managing and treatment of pus-causing infections and wound's recurrent infections in nongovernmental hospitals in Jordan , the risk of development of high resistant drug isolates of wound was high by approximate 22.4% of total infections, and the progression of new types of bacteria specially; *E. coli* ESBL ( *E. coli* carbapenemase XDR), *K. pneumoniae* carbapenemase XDR, *K. oxytoca* ESBL, Vancomycin resistant enterococcus which formed 8.8 while the actual MDR formed 23.2% (see Table 4-B) [21,22].

The high cost and lack of well-trained multi-disciplinary medical personnel, facilities and standardized management protocols are possible contributory factors. Physicians also have an important role in the prevention, early diagnosis and management of infections of wounds with multidrug resistant's or high resistant microbes for chemotherapies. Thus, there is indeed a need to ensure that better focused education and determination the best way to handle and take care about ulcer foot cases on appropriate foot

| Type of pathogen | Number of infections | Percentage |
|------------------|----------------------|------------|
| Acinetobacter spp (MDR) | 2 | 2% |
| S. aureus MRSA | 11 | 11.2% |
| Total number | 14 | 13.3% |

Table 4-C. Multidrug resistant isolates of pus samples
wears, foot care and other harmful practices be intensified among these patients.

4.1 Bacteriological Pattern of Pus Samples

In the present study, a total of 19 different microorganisms were isolated from the participants, with mixed gram-positive and gram-negative species; percentage of 37% gram-positive aerobic bacteria and 63% gram-negative aerobic bacteria, which is need more focus on pus infections in Jordan.

The Predominancy of gram-negative aerobes have been reported also field workers and previous researchers [23]. These differences could be partly due to changes in the causative organisms occurring over time, and the capability of microbes to get more resistance for antibiotics, also might be affected by geographical variations, or the types and severity of infection. Differences in results might be due to the use of a “relatively small number of specimens”, and limited specimen collection techniques (which would fail to exclude superficial or colonizing organisms), the poor handling techniques and poor preservation methods might affect the cultivation of anaerobic organism [24,25,26].

In the present study, a total of 19 different microorganisms were isolated from the participants, most of them were not multi-resistant nor high resistant bacteria for antibiotics by the percentage of 85.7%; E. coli was the most frequent pathogen followed by 21.4% of the total infections then S. aureus (not multi-resistant) by percentage of 14.3% and S. aureus (MDR) by percentage of 12.2% then Nonhemolytic streptococci by percentage of 9.2%, then the others; K. pneumoniae, Streptococcus, agalactiae (group B), Morganella morganii, E. coli ESBL, Streptococcus pyogen, K. oxytoxa ESBL, Acinetobacter spp (MDR), Vancomycin resistant enterococcus, Pseudomonas aeruginosa, P. mirabilis, K. pneumoniae carbapenemase XDR, E. coli carbapenemase XDR, Alpha hemolytic streptococci and Acinetobacter spp consist about 30% of the total infections. Which was corresponded to the global results with mixed gram-positive and gram-negative species; an average of 1:3(7/18) gram-positive aerobic bacteria, with approximate overall 44% of infections per cases. (Table 4-C) [25,27].

The multidrug resistant bacteria in pus isolates formed 13.3% of all infections and the main causative agents were Acinetobacter spp (MDR) which resident in Jordan as a nosocomial infection cause of infection that explain the predominance of it in pus and chronic infections then S. aureus MRSA which is one of the main cause of wound over the world. (Table 4-C) [5,6,9,11,28].

5. CONCLUSION

The global burden from multidrug resistant bacteria highly impacted in wound and pus-causing infections, either in hospital acquired infections or community acquired infections. The main causative agents of recurrent wound infection were Staph. aureus MRSA, E. coli, Pseudomonas aeruginosa, Acinetobacter spp (MDR). Gram-negative bacteria caused the most of infections by more than 67% comparing with gram-positive bacteria.

The infections by High resistant drug of pus isolates and recurrent wound infections had formed approximate more than 22% of the infections specially with new seicies like Acinetobacter spp. (MDR) had been reported, which is new challenge to healthcare providers and infections control commeties in and medical centers in Jordan, more studies should be done at the medical and genome level and in addition to improve treatments procedures, and more regulations and restrictions should be adopted to control antibiotic prescriptions and antibiotics intakes.

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CONSENT AND ETHICAL APPROVAL

Ethical approval was obtained from the Health Research Ethics Committee of the MOH, Jordan. As per international standard or univiersity standard, patients’ written consent has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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