Microbes and Infectious Diseases 2025; 6(1): 342-354

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Occurrence of extremely drug-resistant *Klebsiella* and multidrug-resistant *Enterobacter* species in chronic wound patients

Olaoniye Habeebat Ajigbewu [1], Folasade Muibat Adeyemi *[1], Abideen Akinkunmi Wahab [1], Omotayo Opemipo Oyedara [1], Nana Aishat Yusuf-Omoloye [1], Fiyinfolu Abdulraheem Ajigbewu[2]

1- Department of Microbiology, Faculty of Basic and Applied Sciences, Osun State University, 230212, Osogbo, Nigeria.
2- Department of Community Medicine, Osun State University Teaching Hospital, Osogbo, Nigeria.

ARTICLE INFO

Article history:
Received 29 December 2023
Received in revised form 22 February 2024
Accepted 4 March 2024

Keywords:
Diabetic-foot-infections (DFU)
ESKAPE pathogen
Extremely-drug-resistant (XDR)
Multidrug-Resistance (MDR)

ABSTRACT

Background: Multidrug resistance in Gram-negative pathogens like *Klebsiella pneumoniae* and *Enterobacter* species (spp.) poses a major challenge in the management of chronic wounds, especially in diabetic patients. This study aimed to isolate and identify *Klebsiella* and *Enterobacter* spp. from chronic wound patients in a tertiary hospital in Southwest Nigeria and determine the antibiotic resistance pattern of the recovered isolates. **Methods**: A total of 165 diabetic and other chronic wound swabs were collected and analyzed. Bacterial isolates were identified using API 20E identification kit and confirmed by polymerase chain reaction (PCR) using genus and species-specific primers. Antibiotic susceptibility testing (AST) was done using the Kirby-Bauer disc diffusion method. **Results**: Diabetic foot ulcers were the most prevalent at 32.7%; followed by traumatic wounds (23.6%). Eighty-seven isolates were identified as either *Klebsiella* or *Enterobacter* spp. The predominant species recovered was *Klebsiella* spp. at 75.9% (66/87); while *Enterobacter* spp. was 24.1%. The highest resistance rate was towards nitrofurantoin, ampicillin, and amoxicillin at 97.7% each while the least resistance was to amikacin at 18.4%. Sixteen *Klebsiella* species, six of them *pneumoniae* were found to be extremely drug-resistant (XDR). The multiple antibiotics resistance indices revealed that 98.9% of the isolates were ≥ 0.2. **Conclusions**: The presence of MDR and even XDR strains in chronic wounds is extremely bothersome and is a cause of impending catastrophe in therapeutic options open to clinicians. Hence, an urgent need for a continuous search for viable alternative therapies and a dynamic surveillance program to monitor and tackle this challenge is required.

Introduction

The problem of antimicrobial resistance (AMR) is ranked as the fifth most significant healthcare challenge by the World Health Organization (WHO) [1]. Several pathogens, particularly the ESKAPE pathogen group have exhibited AMR at varying levels. The ESKAPE pathogens are a six-membered group consisting of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*,

DOI: 10.21608/MID.2024.259210.1740
* *Corresponding author:* Folasade Muibat Adeyemi
E-mail address: folasade.adeyemi@uniosun.edu.ng

Pseudomonas aeruginosa, and *Enterobacter* species that have consistently threatened the effectiveness of available conventional antibiotics by devising means to escape their microbicidal effects [2]. The Gram-negative members of this group (*K. pneumoniae*, *A. baumanni*, *P. aeruginosa*, and *Enterobacter* spp.) constitute a public health menace, which is of great concern as alarming levels of resistance have been observed [3].

Klebsiella and *Enterobacter*, both members of the *Enterobacteriaceae* family, comprise species that are primarily associated with nosocomial infections. The most prominent members of the *Klebsiella* genus include *Klebsiella oxytoca*, *Klebsiella ornithinolytica*, *Klebsiella variicola*, and the infamous *K. pneumoniae* [4]. The genus *Enterobacter* consists of about 22 identified species, of which three (*E. cloacae*, *E. aerogenes*, and *E. hormaechei*) are most clinically relevant. Other species include *E. amnigenus*, *E. gergoviae*, and *E. turicensis* [5].

Klebsiella and *Enterobacter* species reside as normal flora in the gastrointestinal and respiratory tracts and on the skin of humans. However, they have been isolated as opportunistic pathogens in immunocompromised patients from both clinical and environmental samples within and outside Nigeria with different levels of multi-drug resistance [6,7].

Klebsiella and *Enterobacter* species utilize different mechanisms of resistance such as the production of enzymes as inhibitors to counter the effects of available antibiotics. Both species can produce extended-spectrum β-lactamases (ESBLs) that hydrolyze the β-lactam ring in β-lactam antibiotic class such as penicillin, cephalosporins, and aztreonam [8,9]. However, ESBL-induced resistance transcends non-inhibition by β-lactam antibiotics alone, as co-resistance to other classes of antibiotics like aminoglycosides and fluoroquinolones, leading to multidrug resistance [8], and resistance to the carbapenems by producing carbapenemase has been reported [9]. In the case of the latter, they are referred to as carbapenem-resistant *Klebsiella* (CRK) and carbapenemase-producing *Enterobacter* spp. respectively. Membrane-associated mechanisms of resistance like up-regulation of efflux pumps, porin defects, and the alteration of the lipopolysaccharide (LPS) layer have been implicated [10,11].

A wound is caused by an impairment in the skin tissue of a host, either through thermal, physical, or microbial damage, and can become chronic when the healing process exceeds four weeks [12]. Chronic wounds mostly occur in immunosuppressed individuals like diabetics, obese, and HbSS patients. Diabetic foot ulcers (DFU), pressure, and venous ulcers, abscesses, and surgical wounds are various forms of chronic wounds. Non-healing wounds can be colonized by many opportunistic pathogens, forming a polymicrobial biofilm community consisting mostly of the Gram-negative members of the ESKAPE pathogen group of organisms [13]. DFUs and other chronic wound infections are difficult to treat, pose a huge burden on the global economy, and are associated with high mortality. They also present the risk of antibiotic resistance development because of the favourable wound environment. Worldwide, the burden of chronic wound infections is estimated to be high with varying prevalence rates [14]. Due to the lack of an organized surveillance system in Nigeria, the accurate prevalence remains unknown. However, a point prevalence as of 2016 was recorded at 11% [15].

Conventionally, bacterial identification is based on morphological and biochemical characterization (rapid kits and automated identification systems). However, misidentification of the different members of the *Enterobacteriaceae* family is frequent, probably due to similarity in many of their biochemical properties and evolution and/or mutation which results in diversities within the species. Within the *Klebsiella* genus, *K. variicola* and *K. pneumoniae* have been frequently misidentified [16], with their nomenclature switched during the identification process. Furthermore, most *Enterobacter* species are usually misidentified as *E. cloacae* or *E. hormaechei* [17]. Hence, the combination of these presumptive phenotypic identification tests with molecular analyses will enhance the accurate and better identification of the species.

Many studies have reported the frequent isolation of members of the ESKAPE pathogen group from chronic wounds, and the necessity for continuous monitoring of the antibiotic resistance patterns of identified pathogens to effectively treat the infections cannot be overemphasized. The aim of this study is, therefore, to accurately identify *Klebsiella* and *Enterobacter* spp. recovered from DFUs and other forms of chronic wound samples in

patients from a Tertiary Hospital and to determine their antibiotic resistance patterns.

Methods

Study design and setting

This is a cross-sectional study involving patients with chronic wounds who attended the General Outpatient Department, as well as in-patients at the male and female surgical wards at Osun State University Teaching Hospital (UTH), Osogbo, Nigeria between November 2021 and April 2023. UTH (with coordinates 7.8707ºN and 4.5699ºE), is a 300-bed government-owned tertiary hospital that serves as a referral center for towns and villages within a forty-kilometre radius of Osogbo township. Participants enrolled for this study were patients who presented with signs of chronic wound infections (delayed ulcer healing ≥ 4 weeks, increasing pain at the wound site, foul odour, gangrenous feet, presence of slough/necrotic tissues, and/or pus). Any wound type falling outside the above classification was excluded from the study. Ethical approval was obtained from the Institutional Ethics Review Committee of UTH, Osogbo (UTH/REC/2023/01/30/739). Informed oral or written consent was sought from the participants or their guardians when ≤ 18 years. A structured questionnaire detailing demographic and other relevant information was also administered to each participant.

Sample collection and processing

A total of 165 wound swabs were collected from patients with chronic wound infections, including post-operative surgical and traumatic wounds, abscesses, and various ulcers **(Figure 1).** The minimum sample size (N) required was estimated using the assumed population proportion formula: $N = \frac{z^2pq}{d}$ where N = minimum sample size required; Z= 95% confidence interval at 1.96; p = the estimated prevalence of chronic wounds of 11% [15], q= 1-p while d = margin of sampling error set at 0.05, along with attrition value of 10%.

Wound samples were collected using sterile cotton-tipped applicators. The edges of the wound sites were cleansed with sterile saline solution, and the swabs were obtained in a rotatory manner as previously described [18]. Samples were inoculated into sterile Tryptone Soy Broth (Oxoid, England), transported immediately to the Microbiology laboratory of Osun State University in coolers with ice packs, and incubated in the transport media at 37±2°C overnight.

Identification of Gram-negative isolates

Phenotypic identification

The cultures were streaked out onto MacConkey agar (Oxoid, England) and incubated at 37±2°C overnight. Preliminary phenotypic identification of the pure *Klebsiella* and *Enterobacter* isolates was based on morphological characterization, Gram-staining, and biochemical characterization using API 20E, and the results interpreted with the APIWEB identification software (Biomérieux, France).

Molecular identification of recovered isolates

DNA extraction and polymerase chain reaction (PCR)

The chromosomal DNA was extracted from presumptively identified *Klebsiella* and *Enterobacter* species by thermal lysis [19]. DNA concentration and purity were evaluated using a NanoDrop-ND-2000 spectrophotometer (Thermo-Fisher Scientific, USA) at 260nm wavelength. Recovered DNA was subjected to Polymerase Chain reaction (PCR) using genus-specific and species-specific primers (Inqaba Biotec, South Africa) to confirm their identification.

Klebsiella pneumoniae and other *Klebsiella* spp. were confirmed by amplifying the 16S−23S rDNA ITS segment using three sets of primers (Pf: 5'-ATTTGAAGAGGTTGCAAACGAT-3'; Pr1: 5'-TTCACTCTGAAGTTTTCTTGTGTTC-3' and Pf/Pr2: 5'-CCGAAGATGTTTCACTTCTGATT-3') [16]. For the *Enterobacter* spp., amplification was done with genus-specific 16SrRNA primer (5'-ATGTCTGGGAAACTGCCTGATG-3', and 5'-CGGGTAACGTCAATAGACAAGG-3') [20].

The PCR protocols were run with the Master Cycler Nexus Gradient 230 (Eppendorf, Germany) using 20µL solution consisting of 10µL of 2× Master-Mix (Biolabs, England), 1µL of 10µM each primer, and 5µL of the DNA template made up with 2µL of DNAse/RNAse free sterile water (BioConcept, USA) in the case of *Klebsiella* spp., and 3µL of DNAse/RNAse free sterile water in the case of *Enterobacter* spp. to the final 20µL volume.

For *Klebsiella pneumoniae/ Klebsiella* spp., the initial denaturation step was carried out at 94ºC for 10 minutes, subsequent denaturation at 94ºC for 30 seconds, annealing at 57ºC for 20 seconds, and extension at 72ºC for 20 seconds. The final extension was set at 72ºC for 10 minutes. For *Enterobacter* spp., initial denaturation was done at 95°C for 5 minutes, followed by 95°C for 30

seconds, annealing at 58°C for 1 minute, and extension at 72°C for 90 seconds, while the final extension was at 72°C for 10 minutes. Both protocols were run for 35 cycles each. Afterward, 10µL each of the amplicons was run on agarose gel stained with 1.0% SafeView Classic at 80V for 60 min along with 100bp DNA Ladder (Biolabs, England), then observed and documented using the UV trans-illuminator E-BOX-CX5 TS imaging system (Vilber, France).

Antibiotic susceptibility testing

This was done using the Kirby-Bauer disc diffusion method to determine the resistance patterns of the bacterial isolates against 15 antibiotics from 9 different classes vis ampicillin, amoxicillin, ampicillin/sulbactam, amoxicillin/clavulanic acid, amikacin, gentamicin, imipenem, meropenem, ceftazidime, cefotaxime, ciprofloxacin, levofloxacin, nitrofurantoin, aztreonam, and sulfamethoxazole-trimethoprim. Pure colonies from overnight cultures of each isolate were inoculated into 5ml of sterile ringer solution and standardized to 0.5 McFarland standard. Thereafter, a lawn of each bacterial suspension was made on sterile Mueller-Hinton agar, allowed to dry and the antibiotic discs were dispensed onto the agar plates using an 8-place Oxoid disc dispenser. The plates were incubated at 37±2°C overnight, and zones of inhibition if any were measured in mm and interpreted with the EUCAST (v12.0) breakpoints table [21].

Statistical analysis

The comparative analysis of collected data was carried out using Microsoft Excel. Frequency distribution and Bar charts were applied for the statistical estimation of the demographic data. The patterns of AMR and the Multiple Antibiotics resistance indices were determined using descriptive statistics.

Results

Demographic characteristics of participants

Altogether, 165 participants comprising 91 (55.2%) males and 74 (44.8%) females who showed clinical signs of chronic wound infection were recruited into this study. The ages of the participants varied between 4 to 90 years, with a mean of 51.6 years; and the highest frequency within the range of 50-59 years**.**

The study recorded more samples from DFUs (32.7%; n=54/165); 32 (59.3%) from male participants. This was followed by traumatic wounds (23.6%; 39/165), with only 1 sample from chronic osteomyelitis (0.6%) (from a 52-year-old hypertensive, albeit educated male with a history of alcohol consumption) **(Figure 2).**

Bacterial profile and characterization of the recovered isolates

Out of 165 samples, 142 (86.1%) showed bacterial growth while 23 (13.9%) had no growth. Overall, 197 Gram-negative bacteria were recovered from the 142 samples, with 53 samples (37.3%) being polymicrobial - two cultures yielding 3 isolates each, 51 samples giving 2 isolates each while the remaining 89 samples (62.7%) yielded single isolates. However, only 44.2% (87/197) were phenotypically identified as *Klebsiella* and *Enterobacter* species. Of these 87 isolates, 26 were from DFUs (29.9%), very closely followed by traumatic wounds (27.6%; n=24/87); while only 1 isolate (1.1%) was recovered from chronic ulcers **(Figure 3).** Upon genotypic screening of the 87 isolates, *Klebsiella* spp. accounted for 50.6% (n=44/87), *Klebsiella pneumoniae* was 25.3% (n=22/87) while *Enterobacter* spp. was 24.1% (n=21/87). In this study, *K. pneumoniae* isolates produced two bands at 260bp with the Pf/Pr2 primer pair and 130bp with the Pf/Pr1 primer pair **(Figure 4a).** Other *Klebsiella* species produced a single band at 130bp or 260bp with either primer pair Pf/Pr1 or Pf/Pr2 **(Figures 4a-4b).** *Enterobacter* spp. was detected at 372bp **(Figure 4c).**

Antibiotics resistance profile

The antibiotic susceptibility pattern of the isolates revealed that one isolate (*Klebsiella* spp. recovered from the surgical wound of a 47-year-old female) was susceptible to all the tested antibiotics while another isolate (another *Klebsiella* spp.) was resistant to all tested antibiotics, and as such classified as extensively resistant (XDR) *Klebsiella* spp. (from a 35-year-old female with a chronic ulcer). The highest resistance was to ampicillin, amoxicillin, and nitrofurantoin at 97.7% each, closely followed by amoxicillin/clavulanic acid (Augmentin) at 93.1%. The least resistance was to amikacin (18.4%), while resistance to imipenem was 27.6%. Several of the recovered isolates exhibited co-resistance to at least 4 of the antibiotics (ampicillin, amoxicillin, amoxicillin/clavulanic acid, and nitrofurantoin), while at least three isolates of *Enterobacter* spp. were resistant to the aminoglycosides, cephalosporins, and monobactam, the last two being β-lactams **(Table 1).** Each of the

species also had at least one isolate resistant to many antibiotics (10 out of 15), and at least one isolate resistant to many antibiotic classes, at least 7 out of 9 classes **(Table 2).** Sixteen isolates (all of them *Klebsiella*) were resistant to all nine classes against which they were tested and also classified as XDR. The Multiple Antibiotics Resistance Indices (MARI) also revealed that 98.9% (86/87) of the isolates had MARI of x ≥ 0.2 with the highest number of isolates (n=22) at 0.5 **(Figure 5).**

Table 1. Antibiotic resistance profile of the recovered *Klebsiella* and *Enterobacter* spp.

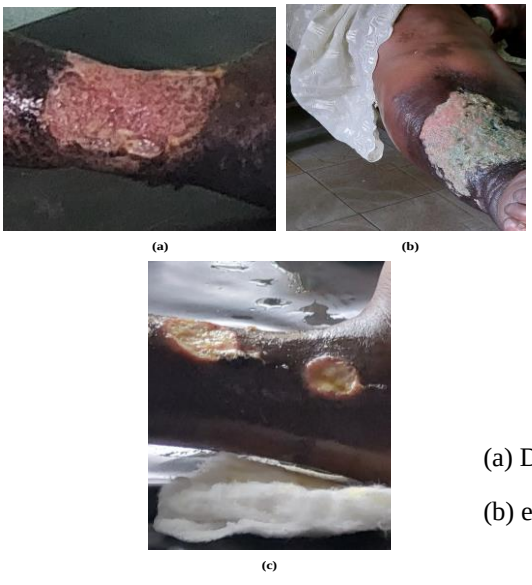
Bacterial Isolate	No tested	Frequency of resistant isolates (%)														
		β-Lactams		β-Lactam inhibitors		Aminoglycoside		Carbapenem		Cephalosporin		Fluoroquinolone		Furans	Monobactam	Sulfonamides
		AMP	AMX	AMS	AUG	AMK	GEN	IPM	MEM	CAZ	CTX	CIP	LEV	NFT	AZT	SXT
Klebsiella pneumoniae	22	21 (95.5)	21 (95.5)	12 (54.5)	22 (100)	2 (9.1)	10 (45.5)	7 (31.8)	19 (86.4)	9 (40.9)	11 (50.0)	10 (45.5)	5 (22.7)	21 (95.5)	12 (54.5)	15 (68.2)
Klebsiella spp.	44	43 (97.7)	43 (97.7)	28 (63.6)	40 (90.9)	11 (25.0)	19 (43.2)	15 (34.1)	36 (81.8)	23 (52.3)	19 (43.2)	26 (59.1)	17 (38.6)	43 (97.7)	24 (54.5)	19 (43.2)
Enterobacter spp.	21	21 (100)	21 (100)	12 (57.1)	19 (90.5)	3 (14.3)	3 (14.3)	2 (9.52)	7 (33.3)	3 (14.3)	5 (23.8)	11 (52.4)	7 (33.3)	21 (100)	3 (14.3)	18 (85.7)
Total	**87**	**85 (97.7)**	**85 (97.7)**	**52 (59.8)**	**81 (93.1)**	**16 (18.4)**	**32 (36.8)**	**24 (27.6)**	**62 (71.3)**	**35 (40.2)**	**35 (40.2)**	**47 (54.0)**	**29 (33.3)**	**85 (97.7)**	**39 (44.8)**	**52 (59.8)**

LEGEND: AMP: ampicillin [10µg]; AMX: amoxicillin [10µg]; AMS: ampicillin/sulbactam [20µg]; AUG: amoxicillin/clavulanic acid [3µg]); AMK: amikacin [30µg]; GEN: gentamicin [30µg]; IPM: imipenem [10µg]; MEM: meropenem [10µg]; CAZ: ceftazidime [10µg]; CTX: cefotaxime [5µg]; CIP: ciprofloxacin [5µg]; LEV: levofloxacin [5µg]; NFT: nitrofurantoin [100µg]; AZT: aztreonam [30µg]; SXT: sulfamethoxazole-trimethoprim [25µg].

Table 2. Multidrug resistance (MDR) profile of the recovered *Klebsiella* and *Enterobacter* spp.

Bacterial isolate (No tested)	Number of antibiotic classes to which isolates were resistant									
	0	1	2	3	4	5	6	7	8	9
Klebsiella pneumoniae (22)	0	0	0	0	5	1	6	2	2	6
Klebsiella spp. (44)	1	0	0	0	6	6	6	10	5	10
Enterobacter spp. (21)	0	0	0	1	4	5	6	5	0	0
Total	**1**	**0**	**0**	**1**	**15**	**12**	**18**	**17**	**7**	**16**

Figure 1. Pictures depicting the chronic wound samples in Osogbo, Nigeria.



(a) Diabetic foot ulcer (DFU); Venous ulcer in a patient with (b) elephantiasis (c) sickle cell disease (HbSS).

Figure 2. Distribution of the different wound types among the patients by gender

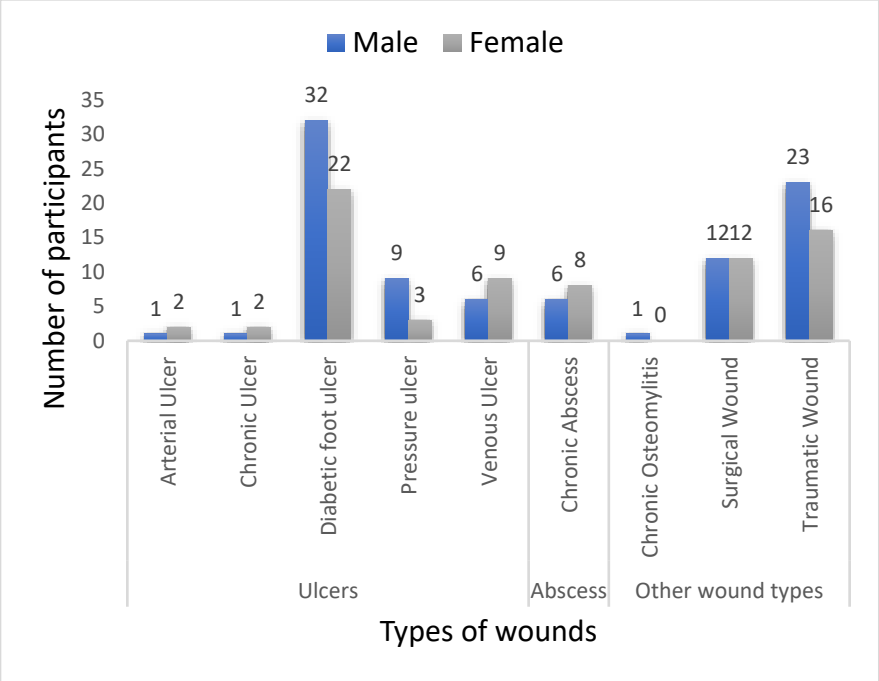


Figure 3. Frequency of occurrence of the Gram-negative bacterial isolates recovered from chronic wounds.

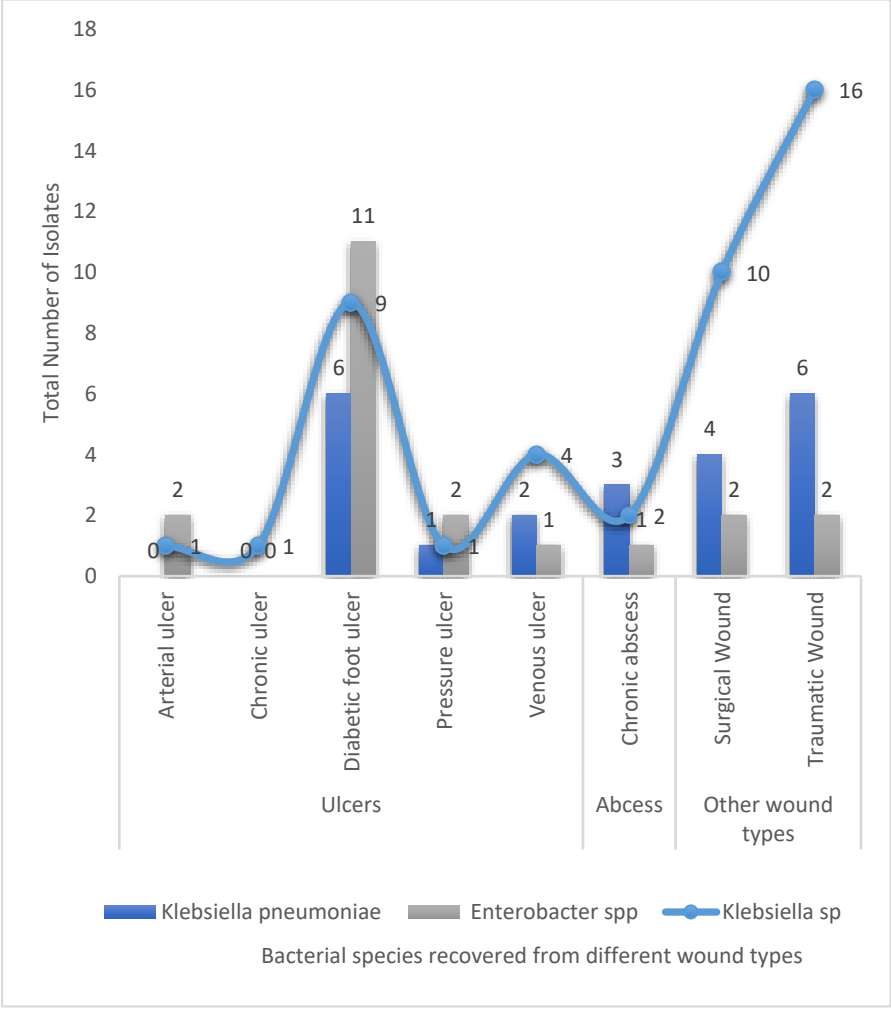
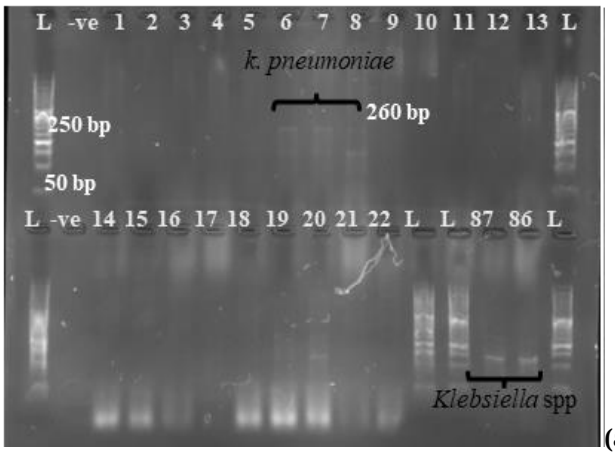
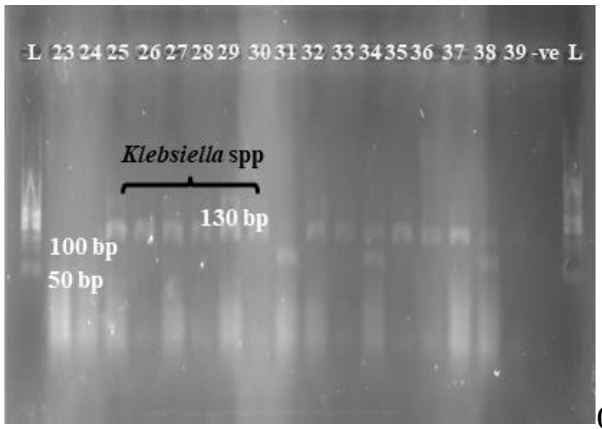


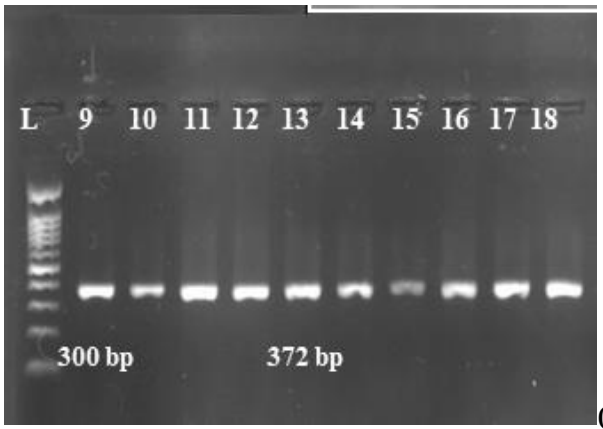
Figure 4. Molecular identification of the recovered isolates.



(a)



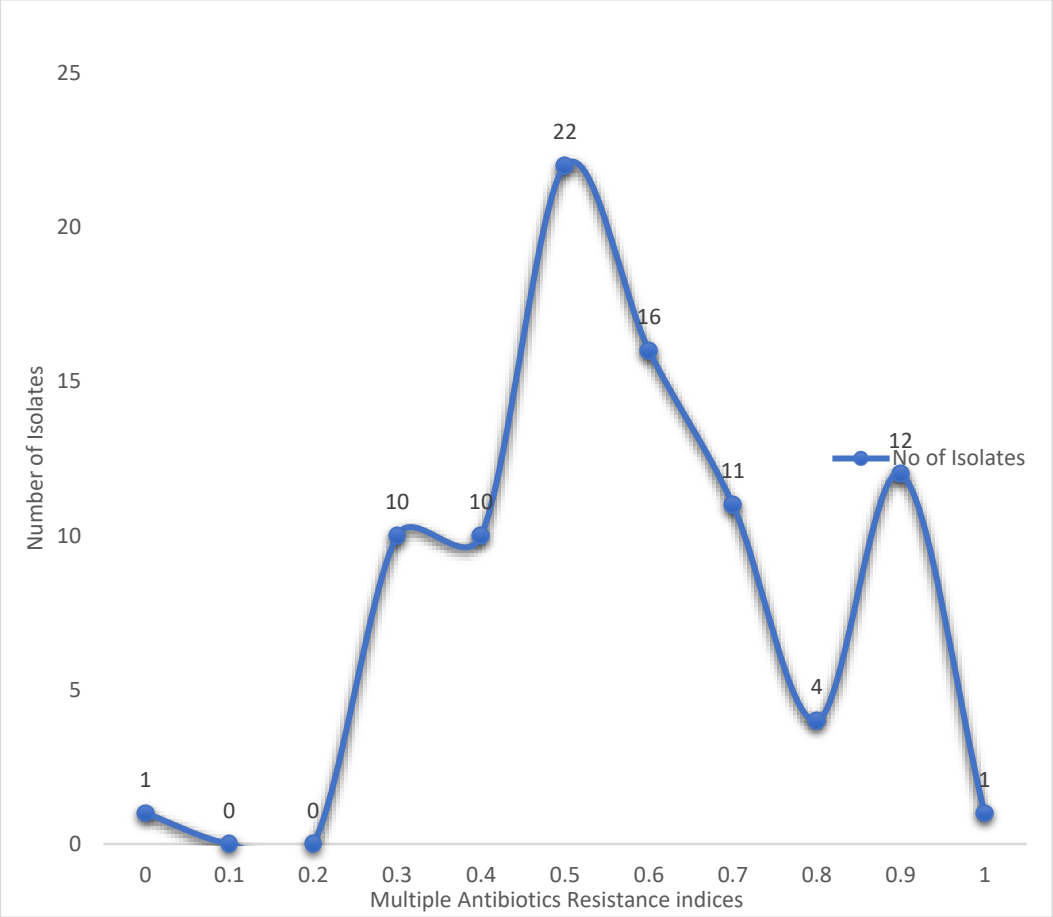
(b)



(c

(a) *Klebsiella pneumoniae* (lanes 6,7,8, and 20) produced double bands at 260bp with Pf/Pr2 and 130bp with Pf/Pr1, whereas isolates 86 and 87 produced a band each at 260bp with Pf/Pr2. (b) *Klebsiella* spp. (lanes 25-30) produced single bands at 130bp with Pf/Pr1 (c) *Enterobacter* spp. (isolates 9-18) produced bands at 372bp.

Figure 5. Multiple antibiotics resistance indices of the recovered isolates.



Discussion

Chronic wounds are responsible for poor quality of life and massive distress in patients with various degrees and duration of the wound resulting in an enormous economic burden on the affected individuals [18]. Bacterial infections by single or mixed species adversely affect chronic wounds, resulting in slowed wound healing, high treatment costs, loss of life or limb, and increased morbidity, particularly in developing countries. Co-morbidities such as diabetes mellitus, hypertension, haemoglobin disorders, and obesity are associated with delayed healing in chronic wounds [22].

This study describes the susceptibility patterns of *Klebsiella* and *Enterobacter* spp. isolated from chronic wound infections. In this study consisting of 165 participants, a higher chronic wound prevalence of 55.2% in male participants, than in female participants, was observed. This result agrees with previous studies conducted in different parts of Nigeria. A higher prevalence of chronic wound infections in males than in females has been reported in Kaduna (62.5%) and Osun states (63.0%) respectively [23,24]. However, contradictory reports also exist [25]. Compared with other African countries, a higher prevalence of chronic wound infections in men than in their female counterparts has also been reported in Ethiopia and Uganda [26,27]. The high incidence of chronic wounds in men can be justified by their occupation as vocations with high occupational hazards like farming, industry, as well as building and construction, are male-dominated, making men more vulnerable to trauma and other work hazards. The male gender is also more adventurous, thereby prone to endangering activities. In this part of the world, it is typical for men to abstain from hospitals in search of treatment unless it becomes life-threatening, heightening the degeneration and chronicity of the wounds.

The chronic wound samples collected were classified into various groups including ulcers (diabetic, pressure, arterial, and vascular ulcers), abscesses, surgical, and traumatic wounds. The different ulcer types accounted for 52.7% (87/165)

of the samples with DFUs having the highest proportion (54/87) on the lower extremities. This correlates well with previous studies [23, 25]. **Pondei et al**. [25] reported that more than a third of their samples (40/101) were from DFU patients.

This study recorded an 86.1% infection rate, with 37.3% of infected samples being polymicrobial, and 62.7% monomicrobial. This isolation rate is higher than the 77.5% from a tertiary hospital in Southwestern Nigeria [28]. However, higher rates within Nigeria of 98.6% [29], and very recently, of 95.7% have been reported in Sierra Leone [30]. This observed high infection rate is probably due to factors such as wound type and management methods, and geographical distributions of the participants.

Molecular identification confirmed most of the isolates (44/87) to be *Klebsiella* species. *Klebsiella* spp. are found copiously in environments, as well as within the gastrointestinal tracts of man and animals [31] which serves as a reservoir for further transmission to infection sites especially in immunocompromised individuals. *Klebsiella pneumoniae* is a prominent opportunistic pathogen responsible for iatrogenic infections and implicated in multidrug-resistant (MDR) wound infections of varying severity [32]. In 2020, fifty-one isolates of *K. pneumoniae* including 26 hypervirulent strains (hvKp) from wound drainage specimens between 2008 and 2017 were characterized in China [33]. Previous studies have established a correlation between colonization and subsequent infection by *K. pneumoniae* in hospitalized patients, attributing this fact to possible host defence impairment due to immunocompromising predisposing factors in the affected individuals [34].

Enterobacter, of the family *Enterobacteriaceae*, is the last member of the ESKAPE pathogen group. It is also found in soil, water, sewage, specific foods, and on human skin as well as the mammalian gastrointestinal tract. *Enterobacter* species are complicit in a wide range of infections and have been recovered from an array of samples such as sputum, wounds, and blood from intensive care units (ICU). Infections due to *Enterobacter* can require extended hospital stays, surgery, non-invasive procedures, and extensive laboratory examinations, in addition to the administration of strong and costly antibiotics. *Enterobacter aerogenes* has been reported to elicit infections in wounds, and the respiratory and urinary tracts, which may degenerate into septic shock in affected patients and increase mortality [6].

The highest frequency of isolates in this study was from DFUs, with *Klebsiella* spp. more prevalent than *Enterobacter* spp. The members of the family Enterobacteriaceae have been reported as the prevalent faction of aerobic Gram-negative rods in DFUs, and overall, *Klebsiella* spp., including *K. pneumoniae* have been more frequently recovered than *Enterobacter* spp. [35], a finding at par with the observed frequencies in the present study. However, various reports exist with a wide diversity of frequently isolated bacterial genera/species from DFUs, including *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter baumanni*, *Morganella morganii*, *Proteus mirabilis*, *Citrobacter*, and *Prevotella* [35]. In a study utilizing culturomics, of 53 recognized and 19 hitherto unidentified bacterial species isolated from DFI patients, *Enterobacter cloacae* was the most prevalent Gram-negative species and third most copious species after *Staphylococcus aureus* and *Enterococcus faecalis* [36]. Using 16S rRNA sequencing, the genus *Klebsiella* and *Enterobacter* were the 3rd and 4th predominant species recovered from DFUs after *Escherichia* and *Proteus* [37].

A myriad of factors have been elaborated to be responsible for the wide variety of species cultured from chronic wounds, and these include although are not limited to duration of infection, geographical locations, history of antibiotic use, and habits of individual patients such as smoking. In addition to these, disparities in infection control/prevention procedures, and the population under study (comorbid illnesses, sex, age) cannot be ruled out as contributing elements [38].

In this study, all *Enterobacter* spp. were resistant (100%) to β-lactam antibiotics and the Furan antibiotics; closely followed by amoxicillin/clavulanic acid (Augmentin) at 90.5% and sulfamethoxazole-trimethoprim at 85.7%. This finding is similar to the report of **Musila et al.** [39] which reported a 100% resistance of all Gram-negative isolates, including *Enterobacter* spp. to β-lactams. However, it contrasts sharply with the pattern of resistance to cephalosporins in our study where we had 14.3% and 23.8% resistance to ceftazidime and cefotaxime respectively, while they had 97.9% resistance to the cephalosporins. However, this can be attributed to the fact that all their selected isolates were carbapenem-resistant. **Wong et al.** [40] reported a high resistance rate to

β-lactams and cephalosporins and also observed a higher frequency of occurrence of resistant strains in chronic than acute wounds.

The resistance rate to gentamicin in the present study was 36.8%, very similar to the 36.4% rate obtained by **Shimekaw et al.** [26] for *Klebsiella* and *Enterobacter* species in their study. Another study reported much higher values of 81.5% and 66.7% gentamicin resistance for *Klebsiella* and *Enterobacter* species respectively [38], much higher than the resistance rate to gentamicin by all *Klebsiella* spp. in our study (43.9%).

There was a distinct variation in the resistance patterns observed to the carbapenem class of antibiotics, as the resistance rate to imipenem was a third of that to meropenem (27.6% to 71.3%) suggesting the efficacy of imipenem over meropenem. This study recorded multiple drug resistance in all but one (98.9%) of the recovered bacterial isolates. The highly susceptible isolate was inhibited by all the tested antibiotics, albeit a strain of *Klebsiella* spp. was resistant to all tested antibiotics. Altogether, sixteen strains of *Klebsiella* (six of them *K. pneumoniae*) were classified as Extremely Drug-resistant (XDR), being resistant to all nine classes of drugs against which they were screened. Extremely drug-resistant organisms are described as those found to be resistant to at least one agent in all but two or fewer antimicrobial categories. All the *K. pneumoniae* isolates exhibited multi-drug resistance to at least 5 antibiotics in 4 different classes, displaying extreme resistance to amoxicillin/clavulanic acid at 100% in comparison to its counterpart ampicillin/sulbactam in the same class (β-lactam/ inhibitors).

Several factors can be attributed to the development of resistance in bacteria. Within the hospital environment, colonizing bacteria are constantly exposed to a variety of antibiotics with different mechanisms of action, leading to the development of resistance. The high MARI values (98.9% ≥ 0.2) in this study are also strongly indicative of an antibiotic-pressurized milieu, which triggers selective pressure. Also, the inappropriate use/misuse of antibiotics such as prophylaxis in animals, wrong prescriptions, over-the-counter purchases, and self-medication; prolonged use of antimicrobial drugs, non-adherence to prescribed dosages, lack of proper antibiotic regulations, and possibly the discharge of antibiotic-laden sewage effluents into the environment could serve as potential causes of the increasing spate of multidrug resistance in bacteria.

The extremely high recovery of MDR strains of *Klebsiella* and *Enterobacter* spp. and even XDR strains of *Klebsiella* in this study is extremely worrisome and could be an ensuing crisis in the management of chronic wounds in the selected tertiary hospitals. This significantly limits the therapeutic options for the treatment of chronic wounds, and as such, it deserves urgent multidisciplinary intervention from public health practitioners, clinicians, the pharmaceutical industry, and other paramedics to institute continuous detection and infection control actions.

However, a limitation of this study that possibly will be undertaken in our succeeding research is the inability to characterize further our isolates using 16SrRNA sequencing, as well as the incapacity to carry out antibiotic resistance gene detection to further validate our results due to constrained financial resources.

Declarations

Source of funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare that no conflict of interest exists.

Ethical approval (include the name of IRB, approval number, and date):

Ethical approval for the study was obtained from the Institutional Ethics Review Committee of UTH, Osogbo (UTH/REC/2023/01/30/739).

Acknowledgments

We hereby acknowledge all staff at the Accident and Emergency as well as the GOPD unit of the UNIOSUN Teaching Hospital (UTH) for their contribution and assistance during the sampling period of this study.

References

1- **World Health Organisation (WHO).** Emergencies. Ten Treats to Global Health in 2019. World Health Organisation, Geneva, Switzerland 2019; p. 1.

2- **Arbune M, Gurau G, Niculet E, Iancu AV, Lupasteanu G, Fotea S, et al.** Prevalence of Antibiotic Resistance of ESKAPE Pathogens Over Five Years in an Infectious Diseases Hospital from South-East of Romania. Infect Drug Res 2021; 14: 2369–2378.

3- **Hijazi S, Visaggio D, Pirolo M, Frangipani E, Bernstein L, Visca P**. Antimicrobial Activity of Gallium Compounds on ESKAPE Pathogens. Front Cell Infect Microbiol 2018; 8: 316.

4- **Rodrigues C, Passet V, Rakotondrasoa A, Diallo TA, Criscuolo A, Brisse S.** Description of Klebsiella africanensis sp. nov., Klebsiella variicola subsp. tropicalensis subsp. nov. and Klebsiella variicola subsp. variicola subsp. nov. Res Microbiol 2019; 170: 165–170.

5- **Davin-Regli A, Lavigne J-P, Pagès J-M.** Enterobacter spp.: Update on Taxonomy, Clinical Aspects, and Emerging Antimicrobial Resistance. Clin Microbiol Rev 2019; 32(4): e00002-19.

6- **Anju VT, Siddhardha B, Dyavaiah M.** Enterobacter Infections and Antimicrobial Drug Resistance. In: **Siddhardha B, Dyavaiah M, Syed A.** (eds) Model Organisms for Microbial Pathogenesis, Biofilm Formation, and Antimicrobial Drug Discovery 2020; Springer Singapore.

7- **Medugu N, Tickler IA, Duru C, Egah R, James AO, Odili V, et al.** Phenotypic and molecular characterization of beta-lactam resistant Multidrug-resistant Enterobacterales isolated from patients attending six hospitals in Northern Nigeria. Sci Rep 2023; 26:13(1):10306.

8- **Adeyemi FM, Oyedara OO, Wahab AA, Akinde SB.** ESβL and MβL production in Gram-negative bacteria isolated from HIV seropositive individuals. Avicenna J Clin Microbiol Infect 2023; 10(1):1-8.

9- **Malekjamshidi MR, Zandi H, Eftekhar F.** Prevalence of Extended-Spectrum β-lactamase and Integron Gene Carriage in Multidrug-Resistant Klebsiella Species Isolated from Outpatients in Yazd, Iran. Iran J Med Sci 2020; 45(1): 23–31.

10- **Masi M, Réfregiers M, Pos KM, Pagès JM.** Mechanisms of envelope permeability and antibiotic influx and efflux in Gram-negative bacteria. Nat Microbiol 2017; 2: 17001.

11- **Ebbensgaard A, Mordhorst H, Aarestrup FM, Hansen EB.** The role of outer membrane proteins and lipopolysaccharides for the sensitivity of Escherichia coli to antimicrobial peptides. Front Microbiol 2018; 9:2153.

12- **Hoversten KP, Lester JK, Stolp AM, Takahashi PY, Verdoorn BP.** Prevention, Diagnosis, and Management of Chronic Wounds in Older Adults. Mayo Clinic Proceedings 2020; 95 (9); 2021-2034.

13- **Di Domenico EG, Farulla I, Prignano G, Gallo MT, Vespaziani M, Cavallo I, et al.** Biofilm is a Major Virulence Determinant in Bacterial Colonization of Chronic Skin Ulcers Independently from the Multidrug Resistant Phenotype. Int J Mol Sci 2017; 18(5):1077.

14- **Martinengo L, Olsson M, Bajpai R, Soljak M, Upton Z, Schmidtchen A, et al.** Prevalence of chronic wounds in the general population: a systematic review and meta-analysis of observational studies. Ann Epidemiol 2019; 29:8-15.

15- **Iyun AO, Ademola SA, Olawoye OA, Michael AI, Oluwatosin OM.** Point Prevalence of Chronic Wounds at a Tertiary Hospital in Nigeria. Wounds (King of Prussia, Pa) 2016; 28(2); 57-62.

16- **Osman EA, El-Amin N, Adrees EAE, Al-Hassan L, Mukhtar M.** Comparing conventional, biochemical and genotypic

methods for accurate identification of Klebsiella pneumoniae in Sudan Access Microbiol 2020; 2(3): acmi000096.

17- **Wu W, Wei L, Feng Y, Xi, Y, Zong Z.** Precise Species Identification by Whole-Genome Sequencing of Enterobacter Bloodstream Infection, China. Emerg Infect Dis 2021; 27(1): 161–169.

18- **Baranoski S, Ayello EA.** Wound Care Essentials: Practice Principles (4th ed.). Lippincott Williams & Wilkins. Stotts, N. A. Wound infection: diagnosis and management. In R. Bryant, & D. Nix (Eds.), Acute and Chronic Wounds: Current Management Concepts 2015; pp.2 83—294.

19- **Adeyemi FM, Yusuf NA, Adeboye RR, Oyedara OO.** Low Occurrence of Virulence Determinants in Vancomycin-Resistant Enterococcus from Clinical Samples in Southwest Nigeria. Int J Infect 2021; (4): e114143.

20- **El-Sayed AKA, Dobara, MIA, El-Shihy, HE.** Simultaneous detection of seven foodborne Enterobacteriaceae pathogens using multiplex PCR. J Egypt Acad Soc Environ Develop 2019; 20 (1): 61-78.

21- **The European Committee on Antimicrobial Susceptibility Testing** Breakpoint tables for interpretation of MICs and zone diameters, Version 12.0 (2022) http://www.eucast.org.

22- **Sen CK.** Human Wound and Its Burden: Updated 2020 Compendium of Estimates. Adv Wound Care (New Rochelle) 2021; 10(5):281-292.

23- **Amaefule KE, Dahiru IL, Okpe IO, Aliyu S, Aruna AA**. Clinico-microbial profile of diabetic foot infections in Zaria, North-West Nigeria. Sahel Med J 2019; 22:28-32.

24- **Ako-Nai AK, Ikem IC, Akinloye O, Aboderin A, Ikem RT, Kassim OD.** Characterization of bacterial isolates from diabetic foot infections in Ile-Ife, Southwestern Nigeria. The Foot 2006; 16: 158-164.

25- **Pondei K, Fente BG, Oladapo O.** Current microbial isolates from wound swabs, their culture and sensitivity pattern at the Niger Delta University Teaching Hospital, Okolobiri, Nigeria. Trop Med Health 2013; 41(2): 49–53.

26- **Shimekaw M, Tigabu A, Tessema B.** Bacterial Profile, Antimicrobial Susceptibility Pattern, and Associated Risk Factors Among Patients With Wound Infections at Debre Markos Referral Hospital, Northwest, Ethiopia. Int J Low Extrem Wounds 2022; 21(2): 182–192.

27- **Wangoye K, Mwesigye J, Tungotyo M, Twinomujuni Samba S.** Chronic wound isolates and their minimum inhibitory concentrations against third-generation cephalosporins at a tertiary hospital in Uganda. Sci Rep 2022; 12(1):1195.

28- **Omoyibo EE, Oladele AO, Ibrahim MH, Adekunle OT.** Antibiotic susceptibility of wound swab isolates in a tertiary hospital in Southwest Nigeria. Ann Afr Med 2018; 17(3): 110–116.

29- **Idowu OJ, Onipede AO, Orimolade AE, Akinyoola LA, Babalola GO.** Extended-spectrum Beta-lactamase Orthopedic Wound Infections in Nigeria. J Glob Infect Dis 2011; 3(3):211-5.

30- **Schaumburg F, Vas Nunes J, Mönnink G, Falama AM, Bangura J, Mathéron H, et al.** Chronic wounds in Sierra Leone: pathogen spectrum and antimicrobial susceptibility. Infection 2022; 50 (4): 907-914.

31- **Dorman MJ, Short FL.** Genome watch: *Klebsiella pneumoniae:* when a colonizer turns bad. Nat Rev Microbiol 2017; 15:384.

32- **Chang D, Sharma L, Dela Cruz CS, Zhang D.** Clinical Epidemiology, Risk Factors, and Control

Strategies of *Klebsiella pneumoniae* Infection. Front Microbiol 2021; 12: 750662.

33- **Zhao Q, Guo L, Wang LF, Zhao Q, Shen DX.** Prevalence and characteristics of surgical site hypervirulent Klebsiella pneumoniae isolates. J Clin Lab Anal 2020; 34(9):e23364.

34- **Meatherall BL, Gregson D, Ross T, Pitout JD, Laupland KB.** Incidence, risk factors, and outcomes of Klebsiella pneumoniae bacteremia. Am J Med 2009; 122: 866–873.

35- **Heravi FS, Zakrzewski M, Vickery K, Armstrong DG, Hu H.** Bacterial Diversity of Diabetic Foot Ulcers: Current Status and Future Prospectives. J Clin Med 2019; 8(11):1935.

36- **Jneid J, Cassir N, Schuldiner S, Jourdan N, Sotto A, Lavigne J-P, et al.** Exploring the microbiota of diabetic foot infections with culturomics. Front Cell Infec Microbiol 2018; 8:282.

37- **Suryaletha K, John J, Radhakrishnan MP, George S, Thomas S.** Metataxonomic approach to decipher the polymicrobial burden in diabetic foot ulcer and its biofilm mode of infection. Int Wound J 2018; 15:473–481.

38- **Hope D, Ampaire L, Oyet C, Muwanguzi E, Twizerimana H, Apecu RO.** Antimicrobial resistance in pathogenic aerobic bacteria causing surgical site infections in Mbarara regional referral hospital, Southwestern Uganda. Sci Rep 2019; 9: 17299.

39- **Musila L, Kyany'a C, Maybank R, Stam J, Oundo V, Sang W.** Detection of diverse carbapenem and multidrug resistance genes and high-risk strain types among carbapenem non-susceptible clinical isolates of target gram-negative bacteria in Kenya. PloS ONE 2021; 16(2): e0246937.

40- **Wong SY, Manikam R, Muniandy S.** Prevalence and antibiotic susceptibility of bacteria from acute and chronic wounds in Malaysian subjects. J Infect Dev Countries 2015; 9(9), 936–944.