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Isolation, Phenotypic Characterization and Prevalence of ESBL-Producing *Escherichia Coli* and *Klebsiella* Species from Orthopedic Wounds in National Orthopedic Hospital Enugu (NOHE), South East Nigeria

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Abstract

The objective of this research work was to isolate, characterize and determine the prevalence of Extended-Spectrum β-Lactamase (ESBL)-producing *E. coli* and *Klebsiella* spp. from orthopedic wounds of patients admitted at National Orthopedic Hospital Enugu (NOHE), South East Nigeria. In this study, 171 bacterial isolates were obtained from 257 orthopedic wound swabs over a period of one year. Sixty nine (69) of the bacterial isolates were identified as *E. coli* while 102 were *Klebsiella* spp based on standard microbiological techniques. The phenotypic screening of the 171 bacterial isolates (*E. coli* and *Klebsiella* spp) for ESBL production was done by disc diffusion method using second and third generation cephalosporins. The ESBL producers were confirmed using double disc synergy test. Susceptibility of the ESBL-producing bacterial isolates to antibiotics was done on Mueller-Hinton agar by Kirby-Bauer disc diffusion methods. Exactly 59.65% and 40.35% of the identified *Klebsiella* spp and *E. coli* isolates respectively were confirmed to be ESBL producers. The bacterial isolates were highly resistant (89%-100%) to ceftazidime, amoxicillin, aztreonam, cefpirome, cefoxitin, cefotetan, and cefotaxime. However, imipenem was the most active antibiotic against the bacterial isolates as they were highly susceptible to this antibiotic (64%-71%). This study has revealed that *E. coli* and *Klebsiella* spp colonize orthopedic wounds. They were also multidrug-resistant with Multiple Antibiotic Resistance Index (MARI) values within the range of 0.20 to 0.85. The increasing prevalence of bacterial resistance to antibiotics has made susceptibility testing a crucial aspect in the treatment of serious bacterial infections. Therefore, there is need for increased surveillance of ESBL-producing organisms as they pose serious threat to successful treatment of infections and exacerbates the problem of antimicrobial resistance in the hospitals, especially in resource poor settings.

Keywords: Orthopedic; Wounds; ESBL; *E. coli*; *Klebsiella* spp

Introduction

Extended-Spectrum β-Lactamases (ESBLs) are bacterial enzymes that hydrolyze and confer resistance to modern cephalosporin antibiotics. They constitute the major mechanism of resistance to second, third and fourth generation cephalosporins (for example: cefuroxime, cefotaxime, ceftriaxone and ceftazidime) [1]. ESBLs have been found in a great number of different bacterial species, but more frequently in *Escherichia coli* and *Klebsiella pneumonia* [2]. There have also been reports of the growing concern of the Enterobacteriaceae and *Pseudomonas* spp producing ESBLs among nosocomial and also community-acquired infections [3]. Wounds occur in countless ways and vary broadly in severity. A wound is a breach in the skin and exposure of subcutaneous tissue following loss of skin integrity. It provides a moist, warm, and nutritive environment conducive for microbial colonization and proliferation [4]. In everyday parlance, wounds typically refer to skin injuries. Clinicians, microbiologists, infection control practitioners, and hospital epidemiologists are concerned about ESBL-producing bacteria because of the increasing incidence of such wound infections, the limitations of effective antimicrobial drug therapy, and adverse patient outcomes [5]. In Nigeria, there have been reports of the reoccurring cases of antimicrobial resistance by most pathogenic organisms against many antibiotics. Moreover, fractional isolated studies establishing the presence of ESBL-producing clinical bacterial isolates from specific localities within the western and eastern part of the country have also been reported [6,7]. ESBL-producing Gram-negative bacteria are emerging and impacting significantly on the management of patients and hospital costs. This study was undertaken to estimate the prevalence of ESBL-producing *E. coli* and *Klebsiella* spp. in orthopedic wounds of patients admitted at National Orthopedic Hospital Enugu. This increasing emergence and development of ESBL-producing bacteria strains which remains a decimating therapeutic impediment clearly point to a present and troublesome problem that could constitute a great deal of menace to futuristic infectious diseases control exercises. Hence, a great deal of attention is required to conduct studies to both identify and fully understand this problem, its cause and scope so as to create an enabling and useful baseline for effective handling of the ESBL threat. Information obtained from this study will contribute towards developing evidence based policy on the rational use of antimicrobial agents, control, prevention, and emergence of multidrug-resistant microbial strains in Nigeria.

Materials and Methods

Sample collection

Two hundred and fifty seven (257) wound samples were obtained from different departments of National Orthopedic Hospital Enugu

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(NOHE). The study population was patients attending the outpatient and inpatient clinics of the hospital. Patients' details (name, age, sex, ward and wound site) were recorded along with the history of infection. Wound samples were collected by swabbing patients' wounds using swab sticks. These samples were immediately transported to the microbiology laboratory unit of Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, Agulu campus in an iced cooler for bacteriological analysis and further tests.

Culturing, isolation, phenotypic characterization and identification of the isolates

The samples were inoculated in a prepared nutrient broth and incubated for 18 h to 24 h at 37°C. Loopful of the inoculated nutrient broth were then cultured by successive streaking on MacConkey agar media (Lab M, UK) for the detection of *E. coli* and *Klebsiella* spp. The suspected *E. coli* and *Klebsiella* spp. isolates were further characterized using conventional/standard microbiology techniques such as colony morphology, Gram-staining, catalase test and other biochemical tests which include oxidase test, indole test, citrate utilization test, H_2S production test, Voges-Proskauer test, methyl red test, urease test and sugar fermentation test [8].

Ethical clearance

Ethical clearance (S/313/IU) was granted by the joint Committee on Human Research Publications and Ethics of the hospital. Approval number is IRB/IIEC NUMBER: S/313/IU.

Preliminary screening for ESBL production

Third generation cephalosporins namely cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), aztreonam (30 µg) and cefotetan (30 µg) were placed on Mueller-Hinton Agar (MHA) inoculated with standardized (McFarland's standard) inoculum. This was incubated at 37°C for 18 h-24 h. Strains of test organisms that were resistant to any of the cephalosporins were suspected to be ESBL producers and were further subjected to Double Disc Synergy Test (DDST) to phenotypically confirm if they are ESBL-producers [9].

ESBL determination by Double Disc Synergy Test (DDST) method

In DDST, synergy was determined between a disc of beta-lactamase inhibitor (amoxicillin (20 µg) and clavulanic acid (10 µg)) and antibiotic disc of third generation cephalosporins (ceftazidime (30 µg) and cefotaxime (30 µg)) placed at a distance of 15 mm apart on a lawn culture of the test isolate on Muller-Hinton Agar. The test isolate was considered to produce ESBL if the inhibition zone size around the antibiotic disc increased above 5 mm in the presence of a beta-lactamase inhibitor disc (amoxicillin (20 µg) and clavulanic acid (10 µg)). This increase occurs because the clavulanic acid inactivates the ESBL produced by the test organism resulting in the formation of extended inhibitory zone [10].

Antibiotics susceptibility testing

The susceptibility patterns of the bacterial isolates were determined by the Kirby and Bauer disc diffusion method as recommended by Clinical Laboratory Standard Institute (CLSI). Each of the isolate was standardized to 0.5 McFarland equivalents and aseptically inoculated on prepared Muller-Hinton agar plates using sterile swab stick. The inoculated plates were allowed to stand for 10 min-15 min. Antibiotic impregnated discs namely; imipenem (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), aztreonam (30 µg), cefoxitin (30 µg), cefpirome (30 µg), amoxicillin (30 µg) and cefotetan (30 µg) (Oxoid, UK) were placed on the inoculated plates using sterile forceps. The plates were incubated at 37°C for 24 h after which the zones of inhibition around each disc were measured to the nearest mm with a metre rule, recorded and interpreted according to the CLSI (2016) guidelines.

Determination of Multiple Antibiotics Resistance Index (MARI)

Multiple Antibiotic Resistance Indices (MARI) of the bacterial isolates were calculated using the technique described by Christopher et al. and Subramani et al. [11,12]. This was calculated as the number of antibiotics to which the tested isolate was resistant to (a), divided by the total number of antibiotics that was tested on the isolates (b).

Results

In this study, 257 orthopedic wound samples were obtained at different patient wards of NOHE as shown in Table 1 above. Table 1 also shows that 171 bacterial isolates (69 *E. coli* and 102 *Klebsiella pneumoniae*) were recovered from the samples.

Table 2 shows that bacterial prevalence was highest in samples collected within the age group of 1 yr to 30 yrs; followed by 31 yrs to 60 yrs, and 61 yrs to 95 yrs being the least. Table 2 also shows that males and females within the age range of 1 yr to 30 and 31 yrs to 60 yrs old had the highest prevalence of orthopedic wounds. Bacterial prevalence was highest in samples collected from civil servants while the least prevalence was observed in samples from Applicants/Housewives as seen in Table 3.

Table 4 shows that bacterial prevalence was higher among in-patients than out-patients. Table 4 also shows that *Klebsiella* spp. was more prevalent (102) than *E. coli* (69) in both in-patients and out-patients. Table 5 shows that bacterial prevalence was highest in samples obtained from legs, followed by samples from hands, while the least prevalence was recorded in samples from chest/neck. Table 5 also shows that orthopedic wounds on the legs are mostly colonized by *Klebsiella* spp. than *E. coli*. The highest bacterial prevalence was observed in samples obtained in accident victims while the least prevalence was observed in samples from patients that had burns as shown in Table 6.

Table 7 shows that the MARI of the ESBL-producing isolates (*E. coli* and *Klebsiella* spp.) ranged from 0.20 to 0.85. Table 8 shows that the *E. coli* isolates in this were resistant to cefpirome (100%), cefotaxime (99%), cefotetan (98%), cefoxitin (97%), ceftazidime

Samples isolated	%	Sections						Total Bacterial examined	%
		GOPD	%	Male ward	%	Female ward	%		
Male=153	59.53	*E.coli* (43)	44.79	*E.coli* (18)	36.73	*E.coli* (8)	32	*E.coli*=69	40.35
Female=104	40.46	*Kleb* (53)	55.2	*Kleb* (31)	63.26	*Kleb* (18)	72	*Kleb* (102)	59.65
Total=257	100	Total=96		Total=49		Total=25		Total=171	100
Key: General outpatient department=GODP; *Kleb=Klebsiella* spp									

Table 1: Samples collected and bacteria isolated.

Age (years)	Sex	Sample	Numbers infected with these organism (%)	
			E. coli [n (%)]	*Klebsiella* spp. [n (%)]
1-30	Male	40	6(8.69)	41(40.19)
	Female	43	17 (24.64)	25 (24.51)
31-60	Male	42	22 (31.88)	19 (18.62)
	Female	45	16 (23.18)	5 (4.90)
61-95	Male	44	8 (11.59)	12 (11.76)
	Female	42	0 (0)	0 (0)
Total		257	69 (100)	102 (100)

Table 2: Age and gender-related bacterial prevalence.

Occupation	Total no. examined [n (%)]	Number of patients infected (%)	
		E. coli [n (%)]	*Klebsiella* spp. [n (%)]
Civil servant	76 (29.57)	19 (27.54)	41 (40.19)
Trader	47 (18.29)	20 (28.98)	25 (24.51)
Students	48 (18.68)	16 (23.19)	19 (18.62)
Applicant/house wives	35 (13.62)	5 (7.25)	5 (4.90)
Artisans	51(19.84)	9 (13.04)	12 (11.76)
Total	257 (100)	69 (100)	102 (100)

Table 3: Occupation-related prevalence of bacterial infection amongst orthopedic wound patients.

Patient's status	Number examined	Number infected (%)	
	[n (%)]	*E . coli* [n (%)]	*Klebsiella* spp. [n (%)]
In-patients	142 (55.25)	33 (47.82)	54 (52.94)
Out-patients	115 (44.74)	36 (52.17)	48 (47.06)
Total	257 (100)	69 (100)	102 (100)

Table 4: Patient's status-related prevalence of bacterial infection.

Location of wound	Number examined [n (%)]	Number infected	
		E. coli [n (%)]	*Klebsiella* spp. [n (%)]
Legs	112 (43.58)	56 (81.15)	76 (74.51)
Hand	104 (40.47)	10 (14.49)	16 (15.69)
Chest/Neck	41 (15.95)	3 (4.35)	10 (9.80)
Total	257 (100)	69 (100)	102 (100)

Table 5: Wound location-related prevalence of bacterial infection.

(96%), aztreonam (95%) and amoxicillin (90%) being the least. Interestingly, table 8 shows that imipenem was the most active antibiotic against the *E. coli* isolates as 71% of them were susceptible to this antibiotic.

Table 9 shows that the *Klebsiella* spp isolates in this study were resistant to ceftazidime (100%), amoxicillin (100%), aztreonam (100%), cefotaxime (97%), cefotetan (96%), cefpirome (95%) and cefoxitin (89%) being the least. Imipenem also proved to be the most active antibiotic against the *Klebsiella* spp. isolates as 64% of them were susceptible to this antibiotic as indicated in table 9.

Discussion

Since wound colonization is most frequently polymicrobial, involving numerous microorganisms that are potentially pathogenic; any wound is at some risk of becoming infected. It is a well known and established fact that in orthopedic wounds, the surgical site infection after implant surgery is a disaster both for the patient and surgeon. This

Cause of wound	Numbers examined [n (%)]	Number infected (%)	
		Escherichia coli [n (%)]	*Klebsiella* spp. [n (%)]
Accident	68 (26.45)	22 (31.88)	34 (33.33)
Burns	24 (9.34)	3 (4.35)	9 (8.82)
Boil	28 (10.89)	6 (8.69)	10 (9.80)
Bullet	36 (14.01)	13 (18.84)	11 (10.78)
Diabetes	32 (12.45)	10 (14.49)	9 (8.82)
Surgery	24 (9.34)	4 (5.79)	7 (6.86)
Ulcer	45 (17.51)	11 (15.94)	22 (21.57)
Total	257 (100)	69 (100)	102 (100)

Table 6: Causes of wound-related prevalence of bacterial infection.

Isolate (*E. coli*)	MARI value	Isolate (*Klebsiella* spp.)	MARI value
CS1	0.71	CS1.75	0.78
CS4	0.78	CS3	0.28
CS5	0.78	CS4	0.71
CS6a	0.20	CS7	0.28
CS6b	0.21	CS13	0.85
CS12	0.57	CS19	0.71
CS15	0.85	CS20	0.20
CS17	0.57	CS22	0.78
		CS25	0.43
		CS36	0.57
		CS38	0.57
Key: CS: Clinical sample			

Table 7: Multiple Antibiotic Resistance Index (MARI) of Isolated ESBL-Positive *K. pneumoniae* and *E. coli* strains.

Antibiotics	Resistance (%)	Susceptible (%)
Ceftazidime	96	4
Amoxicillin	90	10
Cefpirome	100	0
Cefoxitin	97	3
Cefotetan	98	2
Cefotaxime	99	1
Imipenem	29	71
Aztreonam	95	5

Table 8: Antibiotic sensitivity profile of *E. coli* isolated from the orthopedic wounds.

Antibiotics	Resistance (%)	Susceptible (%)
Ceftazidime	100	0
Amoxicillin	100	0
Cefpirome	95	5
Cefoxitin	89	11
Cefotetan	96	4
Cefotaxime	97	3
Imipenem	46	64
Aztreonam	100	0

Table 9: Antibiotic sensitivity profile of *Klebsiella* spp. isolated from the orthopedic wounds.

may lead to increased antibiotic use, prolonged hospital stay; prolonged rehabilitation, morbidity and mortality [13]. Infection of wounds by microorganisms is most often associated with prolonged hospital stay with the attendant risk of acquisition of multiple resistant organisms from medical devices and hospital environment [14]. In this study, 257 orthopedic wound samples were obtained over a one-year period (July 2015-June 2016) at different departments of NOHE (Table 1). A total of one hundred and seventy-one (171) bacterial isolates were recovered

from the samples: 69 were *E. coli* while 102 were *Klebsiella pneumoniae* (Table 1). This study also revealed that bacterial prevalence was highest in samples collected within the age group of 1 yr to 30 yrs; followed by 31 yrs to 60 yrs, and 61 yrs to 95 yrs being the least (Table 2). Bacterial prevalence was also highest in samples collected from civil servants while the least prevalence was in samples from Applicants/Housewives (Table 3). Bacterial prevalence was also high among in-patients than out-patients (Table 4). Our study also showed that bacterial prevalence was highest in samples obtained from legs, followed by samples from hands, while the least prevalence was recorded in samples from chest/neck (Table 5). The highest bacterial prevalence was observed in samples obtained in accident victims while the least prevalence was observed in samples from patients that had burns (Table 6). This trend could be as a result of patients waiting for longer time before seeking medical attention and such situation could lead to heavy growth of bacteria and mixed infection in the wounds. Muhammad showed that *Klebsiella* spp. and *Escherichia coli* were the leading causative agents of orthopedic wound infections [15]. This report is in concord with the results of our study. Our study revealed that *Klebsiella* spp. had the highest infection rate with a frequency of 59.65% while *Escherichia coli* had the least infection rate (40.35%). Our study is in partial agreement with the work of Mehta et al. who reported that *Pseudomonas* spp. was the most common pathogen isolated (51.5%); and closely followed by *Acinetobacter* spp. (14.28%), *Staphylococcus aureus* (11.15%), *Klebsiella* spp. (9.23%) and *Escherichia coli* (2.3%) being the least between the period of 2002 to 2005 [16]. They also reported that *Klebsiella* spp was still the most common pathogen in the burns unit of their study area. Also, this study is in line with the work of Adebayo who reported that orthopedic wounds are more prevalent in people that engaged in field works than indoor jobs. Our study showed that orthopedic wounds on the legs are mostly colonized by *Klebsiella* spp (Table 5). This is in agreement with the work of Adebayo who reported that *Klebsiella* spp and *Staphylococcus aureus* dominated the acute soft tissue infections such as accidents, boils, abscesses and necrotizing infections. Our study shows that males and females within the age range of 1 yr to 30 yrs and 31 yrs to 60 yrs old had the highest prevalence of orthopedic wounds (Table 2). Men had more orthopedic wounds than females probably because of occupational involvement. In this study, the *E. coli* isolates were resistant to cefpirome (100%), cefotaxime (99%), cefotetan (98%), cefoxitin (97%), ceftazidime (96%), aztreonam (95%) and amoxicillin (90%) being the least (Table 8). Interestingly, imipenem was the most active antibiotic against the *E. coli* isolates as 71% of them were susceptible to this antibiotic (Table 8).

The *Klebsiella* spp isolates were resistant to ceftazidime (100%), amoxicillin (100%), aztreonam (100%), cefotaxime (97%), cefotetan (96%), cefpirome (95%) and cefoxitin (89%) being the least (Table 9). Imipenem also proved to be the most active antibiotic against the *Klebsiella* spp. isolates as 64% of them were susceptible to this antibiotic (Table 9). This report shows that the third and fourth generation cephalosporins were ineffective in the treatment of wound colonized infections such as the orthopedic wounds studied. This is in agreement with the work of Muhammed. Our study revealed that 59.6% of the identified *Klebsiella* spp isolates were confirmed as ESBL producers using double disc synergy test. Earlier reports of ESBL producing strains have shown *Klebsiella* spp. as possessing a traditional role in the overall definition and expression of ESBL. Recently, the hospital and community prevalence of ESBL-producing bacteria in South West and South Eastern Nigeria were placed at 7.5% and 4.4% respectively falling slightly short of the values obtained in our study. The MARI of the ESBL-producing isolates (*E. coli* and *Klebsiella* spp.) ranged from 0.20 to 0.85 (Table 7). It has been hypothesized that bacteria with MARI values greater than 0.2 usually emanate from environment where several antibiotics are used or misused. This scenario, apart from determining the ease of passage and acquisition of the resistance traits, also underscores the need for an extensive and constant demographic coverage of the country for an antimicrobial surveillance studies; especially the specialized ESBL-producing bacteria. These data would be useful for present and future intervention exercises [17-19].

Conclusion

This study has revealed that *E. coli* and *Klebsiella* spp. are members of Enterobacteriaceae that colonize orthopedic wounds. The majority of the bacterial isolates in this study were multidrug-resistant as they were resistant to at least two classes of antibiotics. This is also depicted in their MARI values which was higher than 0.2, and hence might have emanated from hospital environment where several antibiotics are used or misused. This study has also established that imipenem is still very active in treating orthopedic wounds bacterial infections. The increased prevalence of microbial resistance to antibiotics has made susceptibility testing a crucial aspect in the treatment of serious bacterial infections. Therefore, there is need for enacting strong antibiotics usage policy in health care settings which depends on the changing or addition of newer antibiotics, their spectrum of activity, pharmacokinetics and pharmacodynamics.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

References

1. Bush K, Jacoby GA, Medeiros AA (1995) A functional classification scheme for beta lactamases and its correlation with molecular structure. Antimicrob Agents Chemother 39: 1211-1233.
2. Lavilla S, Gonzalez Lopez JJ, Miro E, Dominguez A, Llagostera M, et al. (2008) Dissemination of extended spectrum β-lactamase producing bacteria: The food borne outbreak lesson. J Antimicrob Chemother 61: 1244-1251.
3. Ramphal R, Ambrose PG (2006) Extended-spectrum beta-lactamases and clinical outcomes: Current data. Clin Infect Dis 42: S164-S172.
4. Bowler PG, Duerden BI, Armstrong DG (2001) Wound microbiology and associated approaches to wound management. Clin Microbiol Rev 14: 244-269.
5. Paterson DL (2006) Resistance in Gram-negative bacteria: *Enterobacteriaceae.* Am J Med 119: S20-S28.
6. Iroha IR, Oji AE, Esimone CO (2008) Antimicrobial resistance pattern of plasmid mediated extended spectrum beta-lactamase producing strain of *Escherichia coli.* Sci Res Essay 3: 215-218.
7. Albinu I, Odugbemi T, Mee BJ (2003) Extended-Spectrum beta-lactamases in isolates of *Klebsiella species* and *Escherichia coli* from Lagos. Nig J Health and Biomed Sciences 2: 53-60.
8. Cheesbrough M (2004) District Laboratory Practice in Tropical Countries. 2nd Edition, Cambridge University Press, United Kingdom.
9. Chaudhary U, Aggarwal R (2004) Extended Spectrum Lactamases (ESBL): An Emerging Threat to Clinical Therapeutics. Indian J Med Microbiol 22: 75-80.
10. Hirakata Y, Matsuda J, Miyazaki Y, Kamihira S, Kawakami S, et al. (2005) Regional variation in the prevalence of extended-spectrum beta-lactamase-producing clinical isolates in the Asia Pacific region (SENTRY 1998-2002). Diagn Microbiol Infect Dis 52: 323-329.
11. Christopher AF, Hora S, Ali Z (2013) Investigation of plasmid profile antibiotic susceptibility pattern multiple antibiotic resistance index calculation of *Escherichia coli* isolates obtained from different human clinical specimens at tertiary care hospital in Bareilly, India. Annals of Tropical Medicine and Public Health 6: 285-289.
12. Subramani P, Shanmugam N, Sivaraman U, Kumar S, Selvaraj S (2012) Antibiotic resistance pattern of biofilm-forming uropathogens isolated from catheterised patients in Pondicherry, India. Australas Med J 5: 344-348.

13. Edwards C, Counsell A, Boulton C, Moran G (2008) Early infection after hip fracture surgery: Risk factors, cost and outcome. J Bone Joint Surg Br 90: 770-777.

14. Idowu OJ, Onipede AO, Orimolade AE, Akinyoola LA, Babalola GO (2011) Extended-spectrum Beta-lactamase in Orthopedic wound infections in Nigeria. J Global Infect Dis 3: 211-215.

15. Khan MS, Rehman S, Ali MA, Sultan B, Sultan S (2008) Infection in orthopedic implant surgery, its risk factors and outcome. J Ayub Med Coll Abbottabad 20: 23-25.

16. Mehta M, Dutta P, Gupta V (2007) Bacterial isolates from burn wound infections and their antibiograms: An eight year study. Indian J Plast Surg 40: 25-28.

17. Shittu AO, Kolawole D, Ruth Oyedepo EA (2003) Wound infections: In two health institution in Ile Ife, Nigeria. Results of a cohort study. Ostomy Wound Manage 49: 52-57.

18. Esimone CO, Nworu CS, Udeogaranya OP (2007) Utilization of antimicrobial agents with and without prescription by outpatients in selected pharmacies in South-Eastern Nigeria. Pharm World Sci 29: 655-660.

19. Clinical and Laboratory Standards Institute (2007) Performance Standards for antimicrobial Susceptibility testing. 7th Edition, Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, United States.