International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)
'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EVALUATION OF THE *IN VITRO* ACTIVITY OF SOME COMMONLY USED ANTIBACTERIAL AGENTS ON *STAPHYLOCOCCUS AUREUS, PSEUDOMONAS AERUGINOSA* AND *PROTEUS MIRABILIS* ISOLATED FROM WOUNDS FROM TWO HOSPITALS IN EBONYI STATE, SOUTH EAST NIGERIA

ONWA, N. C.[*1], OKEH, C. O[1], OKONKWO. E. C.[2], ILANG, D. C[1]., OKATA-NWALI, D. O[1] AND UDO, M. A[3]

1: Department of Microbiology, Alex-Ekwueme Federal University Ndufu Alike, Ebonyi State, Nigeria

2: Applied Microbiology Department, Ebonyi State University Abakaliki, Ebonyi State Nigeria

3: Alex-Ekwueme Federal Teaching Hospital Abakaliki Ebonyi State, Nigeria

*Corresponding Author: Dr. Onwa NC: E Mail:collins.onwa@ebsu.edu.ng

ABSTRACT

The rise in antimicrobial resistance among pathogenic bacteria species is currently a problem in the clinical setting and treatment of wound infections are increasingly, becoming a challenge. This study assessed, the *in vitro* antibacterial activity of some commonly used antibiotics in Ebonyi State, against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus mirabilis* isolated from patients' wounds. Hundred and fifty (150) wound samples collected from patients attending Alex-Ekwueme Federal University Teaching Hospital, Abakaliki (AE-FETHA) (N=98) and General Hospital Uburu (GHU) (N=52) were used for the study. Structured questionnaires were used to get demographic information about the patients. Wound samples were collected using sterile swab sticks. The bacteria species were isolated and identified by standard microbiological methods. Antibiotics Susceptibility test was performed by Kirby-Bauer-CLSI modified Disc Agar Diffusion method. Results of socio demographic data from the hospitals showed that the greatest number of the wound patients were between 31-45 years of age for both hospitals. Males have more wounds than females for both hospitals too. Wound samples, obtained from accident victims had the highest frequency for AE-FETHA, and

for GHU, followed by burns, surgical and animal bites. The prevalent rate of the three bacteria species from AE-FETHA wound samples were; *Pseudomonas aeruginosa* (46.7%), *Staphylococcus aureus* (46.7%) and *Proteus mirabilis* (6.6%), and their prevalent rate from GHU wound samples were; *Pseudomonas aeruginosa* (64.3%), *Staphylococcus aureus* (28.6%) and *Proteus mirabilis* (7.1%). Result of the antibiotics susceptibility tests, showed that all (100%) of the bacteria species were susceptibile to imipenem. Some *Staphylococcus aureus* isolates were susceptible to the other antibiotics while being totally (100%) resistant to ceftriaxone. *Pseudomonas aeruginosa* showed degrees of susceptibility to the other antibiotics. *Proteus mirabilis* was found to show 50% susceptibility to meropenem and ofloxacine, while being totally (100%) resistant to the other antibiotics. The antibacterial agent; Imipenem, being hundred percent (100%) active against the tested bacteria species, is therefore, recommended as the antibiotics of choice in treating wound patients infected with the bacteria species in the studied locality.

Keywords: Antibacterial agents, Antibiotics, Infection, Patients, Wound

INTRODUCTION:

Wound is a break in the structure of an organ or tissue caused by an external agent [1]. Bruises, grazes, tears, cuts, punctures and burns are all examples of wounds [1]. Wound infections arises from the contamination of injuries sustained on the human body. These injuries results from the breach in the normal continuum of tissues. Wounds provide moist, warm and nutritious environment that is conducive for microbial colonization, proliferation and growth [1, 2]. Therefore, chronic wounds harbour the presence of many microorganisms such as multi-drug resistant bacteria, polymicrobic flora, bacteria, fungi and viruses [3].

The common method used to treat wound infections is by using antimicrobials. Antimicrobials exist as natural, semi-synthetic or synthetic substances. Antibiotics is one of the antimicrobial agents used in treating wound infections.

Antibiotics are chemical substances produced by microorganisms that have the capacity to kill or inhibit the growth of another microorganisms. Antibiotics may be naturally occurring, semi-synthetic or synthetic. The application of antibiotics to treat and prevent diseases have played a critical role in reducing the burden of infectious diseases all over the world [4].

The treatment of wound infections using antibiotics has come under much scrutiny as it have been shown that microbes can acquire multi-drug resistance to some of the antibiotics used in treating human infections [2]. [5] attributed the emergence of antibiotic- resistant bacteria such as Methicillin resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Staphylococcus aureus* (VRSA), Vancomycin-intermediates *Staphylococcus aureus* (VISA) and other drug resistant

species of *Pseudomonas, Proteus* and other bacteria species to enzymatic inactivation of the drug, alteration of target sites of the antibiotics, reduced cellular uptake, decreasing absorption or increasing efflux of the antibiotics and acquisition of the ability to break or modify the antibiotics leading to the depression of the activity of these antibiotics [6. 7]. Antimicrobial resistance by microorganisms to antimicrobial agents is a global public healthcare problem [8], as a result many antibiotics medicines are losing their ability to treat wound infections leading to many mortality and morbidity [8]. The routine surveillance for pathogens and their susceptibility to antibiotics is of paramount importance not only to reinforce strategies for successful wound infection control and management but in the control of antibiotic usage, to stem the emergence and spread of resistant bacteria [8]. Therefore, this study set out to evaluate, the *in vitro* antibacterial activity of some commonly used antibiotics in Ebonyi State, against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus mirabilis* isolated from wounds.

MATERIALS AND METHODS

Well-structured questionnaires were used to obtain information concerning the socio-demographic characteristics of the patients and information from the hospital.

Test organisms

The test bacteria; namely *Stapylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus mirabilis* for this study were isolated from wounds from patients at Alex-Ekwueme Federal Teaching Hospital Abakaliki (AE-FETHA) (n= 98) and General Hospital Uburu (GHU) (n= 52) all in Ebonyi State, Nigeria. Identification and authentication of the isolates were done using standard microbiological methods of cultural, morphological and biochemical tests [9].

Standardization of inocula

The test organisms were standardized by inoculating 5ml normal saline in sterile test tubes with loop full of overnight cultures from nutrient agar slants. The mixtures was shaken to obtain homogenous suspensions. The homogenous suspensions were adjusted to 0.5 McFarland's standard corresponding to 10^8cells/ml.

Antibacterial susceptibility testing.

The antibacterial susceptibility testing was performed using the modified Kirby Bauer disc diffusion method and interpreted according to guidelines of clinical laboratory standards institute [10]. Using a Pasteur pipette, 0.5ml of the standardized inoculum were transferred onto sterile plates of Mueller Hinton agar and spread with a glass spreader. Antibacterial discs used were nitrofurantoin (300μg), nalidixic acid (30μg), meropenem (10μg), imipenem (10μg), aztreonam (30μg), ceftriaxone

(30μg), gentamicin (30μg), ofloxacin (5μg), ciprofloxacin (5μg) and sulphamethoxazole-trimethoprim (25μg). They were placed equidistance to each other using sterile forceps on the Mueller Hinton agar, seeded with each test organism. They were then incubated at 37^0C for 24 hours. After incubation, the diameter of the zone of inhibition against each of the test organisms were measured in mm [11]. The selection of the drugs was based on their availability and prescription frequency in the study area.

RESULTS AND DISCUSSION

In this study, it was observed that the demographic characteristics of the wound patients in the studied hospitals in Ebonyi State varied with respect to age, gender and cause of wounds **(Figure 1)**. For both Alex Ekwueme Federal Teaching Hospital Abakaliki (AE-FETHA) and General Hospital Uburu (GHU), the age range of 31 – 45 years have the highest wound patients at 52.6% for AE-FETHA and 47.4% for GHU. The age range of ≥61 has the least number of wound patients for both hospitals **(Figure 1)**. This finding can be attributed to the fact that most people are active at the ages of 31 – 45 years, thereby exposing them to conditions that can cause wounds, while at the age of ≥61 years, most people have retired, less active and hence less exposed to conditions that can cause wounds. The result of the gender of patients revealed that greater number of patients with wounds in

this study were males, (57.3%) for AE-FETHA and 55.7% for GHU **(Figure 1)**. Wounds caused by accidents had the highest frequency of 54.7%, followed by burns (20%), surgical (15.3%) and animal bites (10%). The demographic statistics that define this study is in consonance with the study made by **[5]**, which showed that wound swab samples collected from male patients were of greater number. This they attributed to the fact that males engage in jobs that are more hazardous than females, thereby exposing them to lesions. Also, the study conducted by **[12]** had similar outcome in terms of age of wound patients but differ in gender. However, in terms of the cause of wound, the study conducted by **[5]** contradicted that of this study were they recorded the highest predominant among ulcer wounds (25.75%).

The prevalence of the isolated bacteria species from the wound samples from AE-FETHA showed that both *Pseudomonas aeruginosa* and *S. aureus* had the same prevalent rate of 46.7% followed by *Proteus mirabilis* with a percentage prevalence of 6.6% **(Figure 2)**. For GHU hospital, *Pseudomonas aeruginosa* had the highest prevalence percentage of 64.3% followed by *S. aureus* with a percentage prevalence of 28.6% and *Proteus mirabilis* with a percentage prevalence of 7.1% **(Figure 3)**. The presence of *Pseudomonas aeruginosa* and *S. aureus* in the wound samples is not

surprising because these organisms are implicated in wound infections. *S. aureus* is a major cause of skin and soft tissue diseases [13]. *Pseudomonas aeruginosa* for example is frequently implicated in burn wounds where they produces systemic diseases [13]. *Proteus mirabilis* is also known to produce focal lesions in debilitated patients and those receiving contaminated intravenous infusions [13]. The findings of this work agrees with the work of [14] and [15], that reported that *Pseudomonas aeruginosa* was more prevalent in wounds than *S. aureus.* But it is in disagreement with the works of [16] and [17] that reported higher prevalent rate of *S. aureus* to *Pseudomonas aeruginosa* in wounds. The higher prevalent rate of *Pseudomonas aeruginosa* over *S. aureus* in the GHU hospital may be attributed to the kind and nature of the wound samples [13].

Antibiotics susceptibility of the test bacteria species from AE-FETHA is presented in **Table 1**. All the seven *S aureus* isolates were susceptible to imipenem (100%), four out of the seven (57.1%) were susceptible to Ofloxacin and three out of the seven (42.9%) were susceptible to meropenem, gentamicin and sulphamethoxazole-trimethropin, while all the seven (100%) were resistant to ceftriaxone. For the seven isolates of *Pseudomonas aeruginosa,* all (100%) were susceptible to imipenem, six out of the seven (85.7%) were susceptible to gentamicin and

three out of the seven (42.9%) were susceptible to ofloxacin and aztreonam. The *Proteus mirabilis* isolate was susceptible to imipenem and meropenem and resistant to all the other antibiotics.

The antibiotics susceptibility of the bacterial species isolated from patients wounds from GHU is shown in **Table 2**. All the four (100%) *S aureus* isolates were susceptible to imipenem and ofloxacin. Two out the four (50%) were susceptible to gentamicin, one out of the four (25%) were susceptible to nalixidic acid, meropenem, and ciprofloxacin and 100% resistant to all the other antibiotics. For *Pseudomonas aeruginosa,* the nine isolated species were all (100%) susceptible to imipenem, eight out of the nine (88.9%) were susceptible to ofloxacine, seven out of the nine (77.8%) were susceptible to ciprofloxacine and gentamicin, four out of the nine (44.4%) were susceptible to nitrofuranton and aztreonam. The *Proteus mirabilis* isolate was susceptible to imipenem and ofloxacine and resistant to all the other antibiotics.

The findings agree with the work of [18] that imipenem was the most active antibacterial agent against *S. aureus*, among the antibiotics used in their study. Ofloxacin and gentamicin were also active *in vitro* against some of the *S. aureus* isolates. In this study, imipenem also inhibited the *Pseudomonas aureginosa,* it was found that ofloxacin and gentamicin are relatively effective

antibiotics against *P aeruginosa* as it inhibited *in vitro* many of the *Pseudomonas aureginosa* isolates from both AE-FETHA and GHU. This findings is in tandem with the work done by **[19]**. *Proteus mirabilis* showed susceptibility to imipenem (100%), Ofloxacin and meropenem. Similar observations has been reported in the study conducted by **[20]** and **[21]** who stated that their effectiveness was as a result of it not being excessively used or abused.
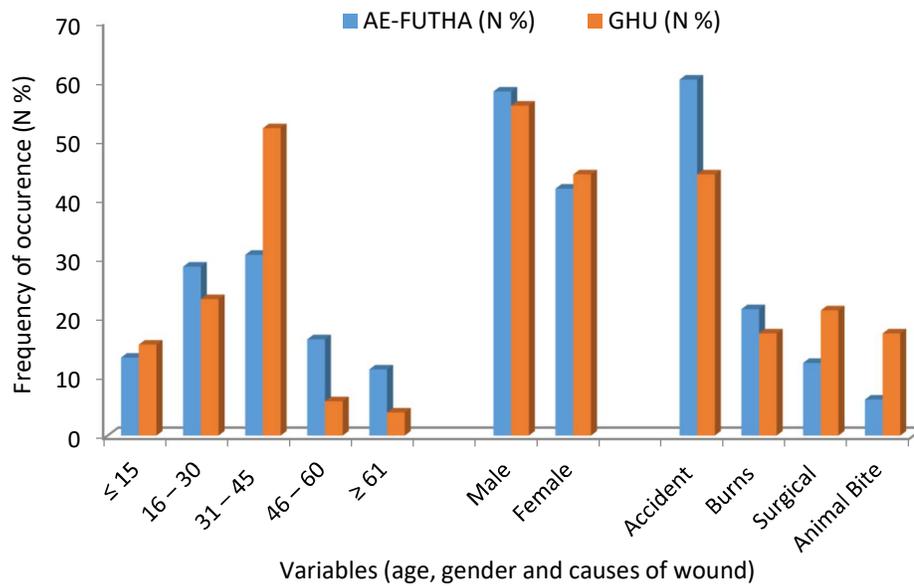

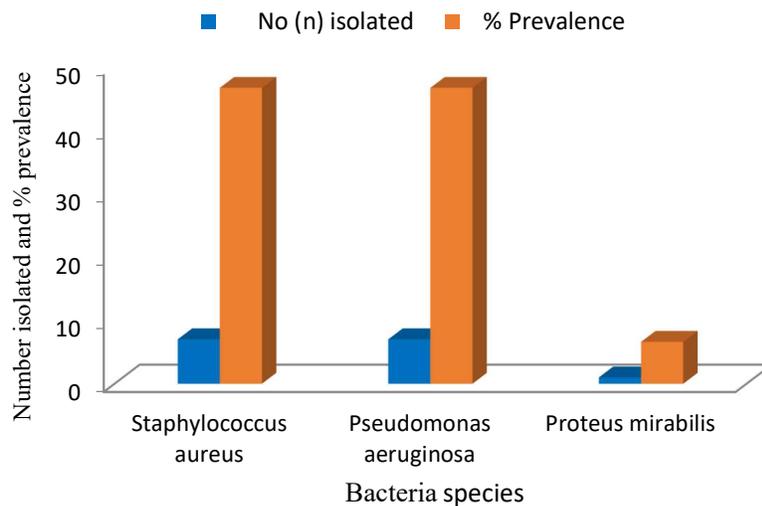**Figure 1: Demographic characteristics of the wound patients**

Figure 2: Percentage prevalence of bacteria species isolated from the 98 wound samples from AE-FETHA
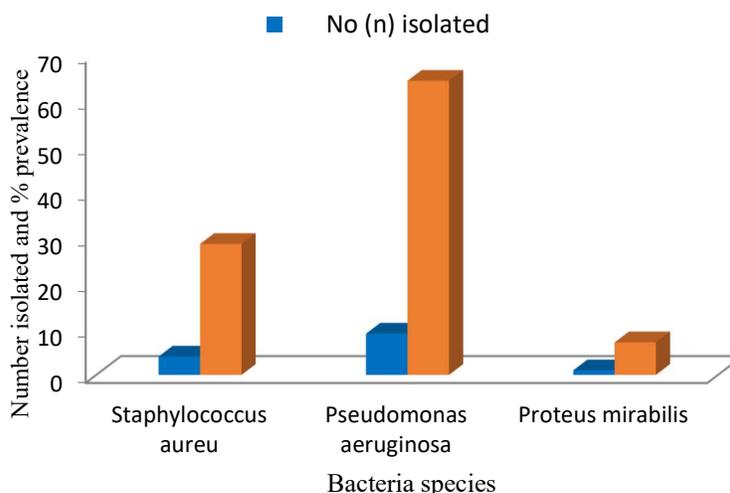
Figure 3: Percentage prevalence of bacteria species isolated from the 52 wound samples from GHU

Table 1: Antibiotics Suceptibility Pattern of the Bacteria from Wounds from Alex-Ekwueme Federal University Teaching Hospital (AE-FETHA) Abakaliki.

		Sa (n=7)	Pa (n=7)	Pm (n=1)
Antibiotics	DP (µg)	Sn(%)	Sn(%)	Sn(%)
Nitrofurantoin	300	1(14.28)	2(28.60)	0(0.00)
Nalidixic Acid	30	1(14.28)	2(28.60)	0(0.00)
Meropenem	10	3(42.90)	1(14.30)	1(100.00)
Imipenem	10	7(100.00)	7(100.00)	1(100.00)
Aztreonam	30	2(28.70)	3(42.90)	0(0.00)
Ceftriaxone	30	0(0.00)	1(14.30)	0(0.00)
Gentamicin	30	3(42.90)	6(85.70)	0(0.00)
Ofloxacin	5	4(57.10)	3(42.90)	0(0.00)
Ciprofloxacin	5	2(28.60)	2(28.60)	0(0.00)
Sulphamethoxazole-Trimethoprim	25	3(42.90)	1(14.30)	0(0.00)

Key: DP=Antibiotic disc potency, Sn (%) number of species susceptible and percentage, Sa=*Staphylococcus aureus*, Pa=*Pseudomonas aeruginosa*, Pm=*Proteus mirabilis*

Table 2: Antibiotics Suceptibility Pattern of the Bacteria from Wounds from General Hospital Uburu (GHU).

		Sa (n=4)	Pa (n=9)	Pm (n=1)
Antibiotics	DP (µg)	Sn(%)	Sn(%)	Sn(%)
Nitrofurantoin	300	0(0.00)	4(44.40)	0(0.00)
Nalidixic Acid	30	1(25.00)	0(0.00)	0(0.00)
Meropenem	10	1(25.00)	3(33.30)	0(0.00)
Imipenem	10	4(100.00)	9(100.00)	1(100.00)
Aztreonam	30	0(0.00)	4(44.40)	0(0.00)
Ceftriaxone	30	0(0.00)	1(11.10)	0(0.00)
Gentamicin	30	2(50.00)	7(77.80)	0(0.00)
Ofloxacin	5	4(100.00)	8(88.90)	1(100.00)
Ciprofloxacin	5	1(25.00)	7(77.80)	0(0.00)
Sulphamethoxazole-Trimethoprim	25	0(0.00)	3(33.30)	0(0.00)

Key: DP=Antibiotic disc potency Sn (%) number of species susceptible and percentage, Sa=*Staphylococcus aureus*, Pa=*Pseudomonas aeruginosa*, Pm=*Proteus mirabilis*

CONCLUSION

Generally, imipenem, Ofloxacin and gentamicin were the most potent antimibacterial agents used in this study against the three bacteria species isolated from patients wounds from the two studied hospitals. Hence imipenem having inhibited the *in vitro* growth of all the *Pseudomonas aeruginosa, S. aureus* and *Proteus mirabilis* should be the drug of choice in the treatment of wound infections, caused by the tested bacteria species from the studied locality.

REFERENCES

[1] Elizabeth A. M. Oxford concise colour Medical Dictionary, Oxford University Press UK. 2010.

[2] Tom, I.M, Agbo, E.B, Umar, A.F., Muhammad, M.I., Askira, M.U., Jidda B.U., Abdullahi, A., and Ali, B.H. Plasmid profile analysis of multi drug resistant Proteus spp, isolated from patients with wound infection in Northeastern Nigeria, Int. J Path Res 2015 1 (4): 1-8.

[3] Adeyinka, A. A., Mshelia, N. and Tolulope, A. Incidence of and risk factors for abdominal surgical site infection in a Nigerian tertiary care centre. *International Journal of Infection Control,* 2018. **3**(4): 13-22.

[4] Street, A., and Bogor, D. Prevalence and sources of contamination of *Escherichia coli* and *Salmonella* spp. in cow milk Dangke, Indonesian Fresh Soft Cheese, Faculty of Veterinary Medicine, Bogor Agriculture University. *American Journal of Veterinary Research*, 2013 **1**(1): 352-356.

[5] Nolan, V. C., Harrison, J., and Cox, J. A. G. (2019). Dissecting the antimicrobial composition of honey. Antibiotics, **8**(251), 1-16.

[6] Rubin, J. E., Wagenarir, J. A., Chirino-Trejo, M., Van Duijkeren, E. and Savelkoul, P. H. M. (2011). Relatedness of methicillin resistant and susceptible *Staphylococcus aureus* from Saskatoon, Canada and the Netherlands and human *Staphylococcus aureus* from Saskatoon, Canada. *PhD Thesis.* **Pp** 92-110.

[7] Ohalete, C. N., Obi, R. K. and EmeaKoroha, M. C. Bacteriology of different wound infection and their antimicrobial susceptibility patterns in Imo State Nigeria. *World Journal of Pharmacy and Pharmaceutical Sciences,* 2019 **1**(3):1155-1172.

[8] Jenkins, R. E. and Cooper, R. Synergy between oxacillin and manuka honey sensitizesmethicillin resistant *Staphyloco ccus aureus* to oxacillin. *Journal of Antim*

icrobial Chemo-therapy, 2012 **6**(7):1405–1407.

[9] Cheesbrough, M. *District laboratory practice in tropical countries* (3rd ed.). United Kingdom: Cambridge University Press. 2010 79-203.

[10] Clinical Laboratory Standard Institute (CLSI). *Performance standards for antimicrobial susceptibility testing*, Wayne, Pennsylvania (21st ed.). 2015 23-26.

[11] Vineetha,N., Vignesh, R. A., and Sridhar, D.. Preparation, Standardization of Antibiotics Discs and study of resistance Patterns for First-Line Antibiotics in Isolates from Clinical Samples, *International Journal of Applied Research* 2015 1(11): 624-631.

[12] Amoran, O. E., Sogebi, A. O. and Fatugase, O. M. Rates and risk factors associated with surgical site infections in a tertiary care center in South-Western Nigeria. *International Journal of Tropical Disease Health*, 2014 **3**(1):25–36.

[13] Geo. F.B., Karen C. Carroll, Janet S. B., Stephen A. M., and Timothy A. M Jawetz, Melnick & Adelberg's Medical Microbiology 26th ed McGraw-Hill Companies USA 2013.

[14] Pandukur, S. G., Sambo, T. T. and Plangnan, A. G. Prevalence of bacterial pathogens associated with wound infections from diabetic out-patients at plateau specialist hospital, Jos, Nigeria. *Open Journal of Bioscience Research*, 2020 **1**(2): 01-11.

[15] Oguntibeju, O. O. and Nwobu, R. A. (2004). Occurrence of *Pseudomonas aerugin osa* in post operative wound infection. *Pakisan Journal Medical Science*, **20**(3):187-191.

[16] Ogba, O. M., Olorode, O. A. and Adie, G. P. Bacterial pathogens associated with wound infections in Calabar, Nigeria. *Journal of medicine,* 2014 **13**(1): 1-33.

[17] Mulugeta, W. and Beyene, G. Postoperat ive nosocomial infections and antimicrob ial resistance pattern of bacteria isolates a mong patients admitted at Felege Hiwot Referral Hospital, Bahidar. *Ethiopia Journal of Health Science,* 2014 **22**(1): 7-18.

[18] Agbakoba N. R., Imouokhome E. I., and Ezeanya-Bakpa, C. C Multi-Drug Resistant Efflux Pumps among Clinical Isolates of *Staphylococcus aureus Nigerian Journal of Microbiology*. 2020 34 (1): 4904 – 4910.

[19] Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y. and Falagas, M. E. Multidrug-resistant, extensively drug-resi stant and pan drug-resistant bacteria: An i

nterna-tional expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection,* 2012 **18**(3): 268 – 281.

[20] Agbagwa, O. E., and Peterside-Frank, N. Effect of raw commercial honeys from Nigeria on selected pathogenic bacteria. *African Journal of Microbiology Research*, 2010 **4**(16):1801-1803.

[21] El-mahmood A. M., Isa A. and Tirmidhi, S. B. Antimicrobial susceptibility of some respiratory tract pathogens to commonly used antibiotics at the Specialist Hospital, Yola, Adamawa State, Nigeria. *Journal of Clinical Medicine and Research*, 2010. **2**(8):135-142.