World Journal of Pharmaceutical ResearCh

Volume 3, Issue 3, 3537-3551. Research Article ISSN 2277 – 7105

ESTIMATION OF RESISTANCE TO HEAVY METALS OF BACTERIAL PATHOGENS CAUSING RESPIRATORY INFECTIONS AMONG WORKERS OF AL-BAIJI OIL REFINERY IN IRAQ

***Mohemid M. Al-Jebouri[1], Abdeljalil H. Al-Samarrai[2], and Riydh A. Abdeljabar[3]**

[1]Department of Microbiology, College of Medicine, University of Tikrit, Tikrit, Iraq.

[2]Department of Biology, College of Education, University of Tikrit Iraq.

[3]Department of Biology, College of Science, University of Tikrit Iraq.

Article Received on 23 January 2014,
Revised on 21 February 2014,
Accepted on 25 March 2014

***Correspondence for Author**

Dr. Mohemid M. Al-Jebouri

Department of Microbiology, College of Medicine, University of Tikrit, Tikrit, Iraq.

profaljebouri@yahoo.com

ABSTRACT

Aims:The most realizable contaminants which are abundant in environment of oil refinery are heavy metals. Some heavy metals like Cu and Zn are essential for bacteria, but even those are highly toxic for all kinds of organisms including bacteria. Resistance to heavy metal, as many authors mentioned, is highly correlated with resistance to antibiotic due to their existence at the same plasmid. Thus, the minimal inhibitory concentration (MIC) of heavy metal is used to investigate the metal tolerance level in certain bacteria.The present study is an attempt to assess the variation of heavy metals resistance among bacterial pathogens isolated from oil refinery workers compares to resistance of the same chemicals among the same bacteria isolated from community patients in hospitals. **Methodology**: With regard to the refinery workers, from October 2008 to September 2009, two hundred samples were taken from patients with upper respiratory tract infections (URTIs) and lower respiratory tract infections (LRTIs). They were employed in the North Refineries Company in Al-Baiji town. Those patients were from different residences, either rural or urban inhabitants, and some of them were living in the resident near by the refinery. Equal number of samples was also taken from patients hospitalized in Tikrit teaching hospital from May 2009 to October 2009 as control samples for comparison. Minimal inhibitory concentration (MIC), under defined test conditions inhibits the visible growth of the bacterium being investigated. MIC values are used to determine susceptibilities of bacteria to heavy metals including cadimium, copper, nickel, lead,vanadium and zinc.

Results: The susceptibility of these groups was tested against the six heavy metals (cadmium, copper, nickel, lead, vanadium and zinc) as a minimal inhibitory concentration (MIC). Results showed that isolates of refinery recorded 73% from the highest values of MICs, while isolates of hospital recorded only 27% of them. **Conclusions**: Inhalation of metal particles might be more dangerous than other routes like digestive system or skin. Workers with low experience, associated with short employment, constituted the highest percentage of infections. Bacteria isolated from patients of the refinery were more virulent due to their relative high metal resistance comparing with those of hospital.

Keywords: Oil refinery, heavy metals, resistance, respiratory infections, bacteria**.**

1.INTRODUCTION

Steadily existing of particles in air inhaled by workers make wide changes in equilibration of biochemical reactions in human body. The most sensitive portion is the respiratory system due to the direct exposure to the toxicants, therefore, human body begins to response negatively to these factors. Many studies show that people exposed to industrial pollution have to develop symptoms of respiratory tract infection [1]. There are many respiratory infections such as tonsillitis, bronchitis, pharyngitis and chest infections occur as a result of negligence and failing to comply with the legislations and laws of work [2].

For bacteria, either chemical or physical changes affected the criteria of any environment where they exist, can result in dramatic changes in their properties, under this pressure, bacteria have to tolerate these excessive concentration of such contaminants [3]. The most realizable contaminants which are abundant in environment of refinery are heavy metals. Environment of oil refinery is a high metal polluted site due to the presence of such metals within the structure of the oil itself like Fe, Mn, Co, Ni, Zn, Cu, Pb, Cr, [4]. Some heavy metals like Cu and Zn are essential for bacteria, but even those are highly toxic for all kinds of organisms including bacteria. Eventually, bacteria have to modify their genetics to tolerate the new concentrations [4]. From the medical point of view, that means a high virulent bacteria which need different chemical therapy [5].

Resistance to heavy metal, as many authors mentioned[3], is highly correlated with resistance to antibiotic due to their existence at the same plasmid. Thus, the minimal inhibitory concentration (MIC) of heavy metal is used to investigate the metal tolerance level in certain bacteria [6].The present study was an attempt to assess the heavy metals resistance of human pathogens associated with workers in oil refinery of Al-Baiji,Iraq.

2.MATERIALS AND METHODS

2.1 Patients

With regard to the refinery workers, from October 2008 to September 2009, two hundred samples were taken from patients with upper respiratory tract infections (URTIs) and lower respiratory tract infections (LRTIs). They employed in the North Refineries Company in Al-Baiji town. Those patients were from different residences, either rural or urban inhabitants, and some of them were living in the resident near by the refinery. The range of age of patients in the refinery was from 16- 65 years old.The patients randomly selected from oil refinery were treated at home,i.e not hospitalized. The workers were employed for different times in oil refinery ranged from 3 to 22 years.According to the nature of such plants, all cases studied were males except one case. The kinds of work for those patients were ranged from low risk job like office job up to high risk like those dealing with hazardous chemicals or working in high contaminated areas [7].

Equal number of samples was also taken from patients hospitalized in Tikrit teaching hospital from May 2009 to October 2009 as control samples for comparison. Patients of hospital were different from those of refinery because about 24% of them were females and the range of age was different, also due to the probability of younger visitors to the hospital.The patients selected from hospital did not work at in oil refinery and they had various civilian jobs and/or unemployed. For both refinery and hospital, swabs from URTIs: (tonsillitis, laryngitis, sore throat and pharyngitis) were transported to the laboratory by using transport medium swabs, and sputa from LRTIs (bronchitis and pneumonia) were transported in screw -capped vials containing Cary-Bliar medium[8]. The samples were taken according to the diagnosis of specialist physician after washing the mouth three times to eliminate much amounts of mouth normal flora [9].

Swabs taken from upper respiratory tract and a loopful of each sputum sample taken from lower respiratory tract were as soon as arrived the laboratory, cultured on doublicated plates of culture media recommended for primary isolation of suspected pathogens to cause a respiratory infection. The inoculated plates were Blood agar, Chocolate agar and MacConkey's agar. Blood agar and Chocolate agar were incubated in a candle jar to enhance growth [10], while the other two plates were incubated aerobically. All the doublicated plates were incubated at 37 C^0 for 18-24 hours [11]. Plates were examined for the characteristics and gases requirements of the isolated colonies and recorded. Negative plates were re-incubated for further 24 to 48 hours with daily checking before discarding [12]

2.2 Identification

The identification of different isolates was carried out according to the conventional methods described by Mahon *et al*[9], Cruickshank *et al*. [10] and Cowan and steel[13].All isolates were identified utilizing morphological as well as biochemical differential tests as classified by Cowan and Steel[13] and confirmed by API systems(France) according to manufacturer instructions.

2.3 Determination of minimal Inhibitory concetration (MIC) of heavy metals of bacteria causing respiratory tract infections Minimal inhibitory concentration (MIC), under defined test conditions inhibits the visible growth of the bacterium being investigated. MIC values(Table 1) were used to determine susceptibilities of bacteria to heavy metals [14].

Table 1: Heavy Metals Used in Minimal Inhibitory Concentration Testing of Bacteria Isolated from Respiratory Infections.

METALS	ATOMIC WEIGHT	CHEMICAL FORMULA	MOLECULAR WEIGHT	CONC. (mM)	MANUFACTURER
Cadmium (Cd^{+2})	112.40	$CdCl_2$	183.31	0.04 to 4.0	British Drug House (BDH)
Copper (Cu^{+2})	65.37	$CuCl_2$	134.45	0.04 to 5.5	British Drug House (BDH)
Nickel (Ni^{+2})	58.71	$NiCl_2.H_2O$	237.71	0.04 to 6.0	British Drug House (BDH)
Lead (Pb^{+2})	207.19	$Pb(CH_3COO)_2.3H_2O$	379.33	0.04 to 16	British Drug House (BDH)
Vanadium $)^{+2}$(V	50.942	$VOSO_4.H_2O$	180.942	0.04 to 15	HIMEDIA
Zinc (Zn^{+2})	65.37	$ZnCl_2$	136.28	0.04 to 12	Hopkin and Williams

Statistical Analysis

Statistical analysis was applied by using the Chi square and t- test (the means of treatments were compared by Duncan's Multiple Range test), under the level of significance ($p \leq 0.05$).

3.RESULTS AND DISCUSSION

Owing to the high medical importance for the heavy metal-tolerant bacterial strains [15], therefore, this study focused mostly on the highest values of MIC. Tables 2, 3, 4, 5, 6 and 7, show the comparison between highest values of MIC of the refinery and those of the hospital for the heavy metals cadmium, copper, nickel, lead, vanadium, and zinc, respectively. Also these tables summarize results of MIC of heavy metals in a way thought to be more expressive to the effect of heavy metals on bacterial activity in contaminated ambient.

Due to the 16 bacterial groups classified in this study, besides the 6 heavy metal studied, that yielded in 24%(96/400) cases of comparison between isolates of the refinery and those of the hospital and the other samples(76%) from both sites revealed negative cultures. Highest values of MIC for the refinery exceeded those of the hospital which constituted 73%. On the other hand, the highest values of MIC of hospital isolates constituted 27% only. It was concluded that *Pseudomanas* spp. Isolated from oil refinery were more resistant to cadimium than the same bacterial species isolated from hospital patients(Table 2).Moreover,cadimium assessment in the present study revealed different levels of resistance among bacteria studied weither from refinery or hospital and this might be due to exposure time and concentration of this metal by these bacteria[17,18].On the other hand,Table 3 shows that *Klebsiella* and *Pseudomonas* revealed the highest resistance to copper in refinery but *Citrobacter* from the hospital patients showed the highest resistance to the same metal.The present result can explain the selectve pressure phenomenon affecting various bacterial types by various heavy metals.This result presented here revealed a clear indication that the environment of refinery- as a contaminated area- takes an important role to make bacterial strains more resistant for heavy metals as compared to those from less contaminated sites. Consequently, they become more virulent, as many workers concluded [16,17]. Furhthermore,*Staphylococcus aureus* revealed similar resistance level(6 μg/ml) to lead heavy metal among both refinery and hospital isolates(Table 4).This might be due to heavy pollution of the local environment by different oil derivatives like gasoline coming from petrol station and their various domestic usage. Nevertheless, Iwegbue *et al.* [21] showed that lands contaminated with high rates of oil spillage contain high concentration of heavy metals like Cd, Cu, Cr, Pb, Mn and

Ni.Surface runoff from refining operations usually had a low pH and contains high levels of combustion metals such as iron, manganese, zinc, copper, nickel, vanadium and cobalt. The of fossil fuels pollutes the atmosphere with metals particles which they eventually settle in land surface. Storm water runoff often contains metals from roadways and atmospheric fallout [22,23].

As for the cases when bacterial group from both the refinery and the hospital recoded an equal maximum value of MIC for vanadium (also in case of superiority of hospital MIC values,Table 5), this case could be explained either by a probability of exposure to continuous pollutants by a patient of the hospital, or due to the work type they are in contact with. The continuous exposure has produced high resistance to the heavy metals and antibiotic as well [6]. Some patients lacking health education suffered from deterioration of health conditions due to his/her continuous exposure to the pathogen and abuse of drug [18].However,zinc assessment showed that the highest level(34 µg/ml) of resistance was seen among *Streptococcus pyogenes* isolated from refinery patients(Table 6) and among *Staphylococcus aureus* isolated from hospitalilzed patients(5.5µg/ml). From the environmental point of view, microorganisms, especially bacteria, could be the key to reduce the threats of heavy metal contamination. Some bacterial species are capable of exhibiting high MICs. For example, studies of Yilmaz [19] indicated that a newly characterized *Bacillus circulans* strain exhibited high minimal inhibitory concentration values for heavy metal ions. Hence, the bacterial species exhibiting high MIC could be of great significance since they could possibly be applied for bioremediation in heavy metal contaminated environment.

Table 2:Maximum Values of Minimal Inhibitory Concentration (MIC) for Cadmium .

SITES		BACTERIAL GROUPS															
		Actinomycetales	*Bacillus* spp.	*Bordetella bronchiceptica*	*Citrobacter* spp.	CONs	*Escherichia coli*	*Haemophilus influenzae*	*Klebsiella* spp.	*Moraxella catarrhalis*	Neisseriaceae	Other species of Enterobacteriaceae	Other species of Lactobacillales	*Pseudomonas* spp.	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Streptococcus pyogenes*
R	No. & (%)	1(14.2)	1(33.3)	1(100)	1(25)	2(15.3)	15 (93.7)	1(5.2)	1(16.6)	4(100)	1(12.5)	3(50)	5(19.2)	1(14.2)	1(5.8)	7(31.8)	22(35.4)
	Conc.(μg/ml)	2.5	0.8	0.9	0.4	3.5	0.5	3.5	3.0	1.0	0.5	3.0	2.0	3.5	4.0	2.0	2.0
H	No . & (%)	3(60)	1(100)	-	4(16)	2(6.4)	1(100)	1(50)	4(66.6)	2(100)	-	2(28.5)	2(9)	3(42.8)	2(5.5)	2(16.6)	6(11.5)
	Conc.(μg/ml)	0.4	0.09		0.9	3.5	1.5	0.2	1.5	0.6		2.0	3.0	2.0	0.3	3.0	1.0

R, isolates of refinery; H, isolates of hospital;CONs,coagulase-negative staphylocci.

Table 3:Maximum Values of Minimal Inhibitory Concentration (MIC) for Copper.

SITES		BACTERIAL GROUPS															
		Actinomycetales	*Bacillus* spp.	*Bordetella bronchiceptica*	*Citrobacter* spp.	CONs	*Escherichia coli*	*Haemophilus influenzae*	*Klebsiella* spp.	*Moraxella catarrhalis*	Neisseriaceae	Other species of Enterobacteriaceae	Other species of Lactobacillales	*Pseudomonas* spp.	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Streptococcus pyogenes*
R	**No. & (%)**	**2(28.5)**	**2(66.6)**	**1(100)**	**4(100)**	**2(15.3)**	**1(6.2)**	**2(10.5)**	**1(16.6)**	**2(50)**	**8(100)**	**2(33.3)**	**2(7.6)**	**2(28.5)**	**17(100)**	**5(22.7)**	**7(11.2)**
	Conc.(μg/ml)	**0.4**	**0.5**	**0.4**	**4.5**	**3.0**	**4.0**	**3.5**	**5.5**	**1.0**	**4.5**	**5.5**	**4.5**	**5.5**	**4.5**	**4.5**	**3.5**
H	**No . & (%)**	**4(80)**	**1(100)**	**-**	**1(4)**	**2(6.4)**	**1(100)**	**1(50)**	**1(16.6)**	**2(100)**	**-**	**4(57.1)**	**10(45.4)**	**3(42.8)**	**27(75)**	**4(33.3)**	**8(15.3)**
	Conc.(μg/ml)	**0.5**	**0.3**		**5.5**	**3.0**	**4.5**	**0.7**	**5.0**	**0.8**		**3.5**	**0.7**	**4.0**	**4.5**	**4.5**	**2.0**

R, isolates of refinery; H, isolates of hospital;CONs,coagulase-negative staphylococci.

Table 4: Maximum Values of Minimal Inhibitory Concentration (MIC) for Nickel

SITES		BACTERIAL GROUPS															
		Actinomycetales	*Bacillus spp.*	*Bordetella bronchiceptica*	*Citrobacter spp.*	CONs	*Escherichia coli*	*Haemophilus influenzae*	*Klebsiella spp.*	*Moraxella catarrhalis*	Neisseriaceae	Other species of Enterobacteriaceae	Other species of Lactobacillales	*Pseudomonas spp.*	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Streptococcus pyogenes*
R	No. & (%)	1(14.2)	1(33.3)	1(100)	2(50)	3(23)	2(12.5)	2(10.5)	1(16.6)	1(25)	1(12.5)	1(16.6)	6(23)	2(28.5)	4(23.5)	12(54.5)	6(9.6)
	Conc.(µg/ml)	0.5	3.0	0.4	2.5	3.0	2.0	2.5	3.5	1.5	3.0	3.5	5.5	3.0	6.0	5.5	3.0
H	No . & (%)	1(20)	1(100)	-	4(16)	1(3.2)	1(100).	2(100)	3(50)	1(50)	-	3(42.8)	4(18.1)	4(57.1)	2(5.5)	2(16.6)	4(7.6)
	Conc.(µg/ml)	2.5	0.1		3.5	2.0	0.9	0.05	0.5	0.7		0.4	1.0	2.0	6.0	2.5	3.0

R, isolates of refinery; H, isolates of hospital;CONs,coagulase-negative staphylococci.

Table 5: Maximum Values of Minimal Inhibitory Concentration (MIC) for Lead .

SITES		BACTERIAL GROUPS															
		Actinomycetales	*Bacillus* spp.	*Bordetella bronchiceptica*	*Citrobacter* spp.	CONs	*Escherichia coli*	*Haemophilus influenzae*	*Klebsiella* spp.	*Moraxella catarrhalis*	Neisseriaceae	Other species of Enterobacteriaceae	Other species of Lactobacillales	*Pseudomonas* spp.	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Streptococcus pyogenes*
R	**No. & (%)**	**4(57.1)**	**3(100)**	**1(100)**	**3(75)**	**1(7.6)**	**4(25)**	**5(26.3)**	**1(16.6)**	**1(25)**	**3(37.5)**	**1(16.6)**	**3(11.5)**	**3(42.8)**	**7(41.1)**	**3(13.6)**	**10(16.1)**
	Conc.(µg/ml)	**12**	**3.0**	**0.1**	**14**	**4.5**	**15**	**5.0**	**14**	**2.5**	**2.0**	**15**	**15**	**4.0**	**6.0**	**15**	**14**
H	**No . & (%)**	**3(60)**	**1(100)**	**-**	**8(32)**	**4(12.9)**	**1(100)**	**2(100)**	**4(66.6)**	**2(100)**	**-**	**2(28.5)**	**3(13.6)**	**3(42.8)**	**12(33.3)**	**3(25)**	**6(11.5)**
	Conc.(µg/ml)	**7.0**	**0.1**		**14**	**0.9**	**13**	**0.5**	**13**	**0.5**		**11**	**15**	**2.5**	**1.0**	**16**	**12**

R, isolates of refinery; H, isolates of hospital;CONs,coagulase-negative staphylococci

Table 6: Maximum Values of Minimal Inhibitory Concentration (MIC) for Vanadium .

SITES		BACTERIAL GROUPS								
		Actinomycetales	*Bacillus* spp.	*Bordetella bronchiceptica*	*Citrobacter* spp.	CONs	*Escherichia coli*	*Haemophilus influenzae*	*Klebsiella* spp.	*Moraxella catarrhalis*
R	No. & (%)	2(28.5)	1(33.3)	1(100)	1(25)	2(15.3)	14(87.5)	2(10.5)	3(50)	2(50)
	Conc.(µg/ml)	6.0	3.0	0.05	10	2.5	5.5	4.5	14	0.8
H	No . & (%)	1(20)	1(100)	-	2(8)	2(6.4)	1(100)..	1(50)	2(33.3)	2(100)
	Conc.(µg) /ml)	4.0	0.1		10	0.8	15	1.5	6	0.5

SITES		Neisseriaceae	Other species of Enterobacteriaceae	Other species of Lactobacillales	*Pseudomonas* spp.	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Streptococcus pyogenes*
R	No. & (%)	4(50)	3(50)	4(15.3)	1(14.2)	5(29.4)	3(13.6)	4(6.4)
	Conc.(µg/ml)	1.0	10	13	4.0	6.0	10	13
H	No . & (%)	-	1(14.2)	5(22.7)	3(42.8)	6(16.6)	5(41.6)	11(21.1)
	Conc.(µg) /ml)		8.0	12	2.5	2.0	4.5	2.0

R, isolates of refinery; H, isolates of hospital;CONs,coagulase-negative staphylococci.

Table 7: Maximum Values of Minimal Inhibitory Concentration (MIC) for Zinc

SITES		BACTERIAL GROUPS															
		Actinomycetales	*Bacillus* spp.	*Bordetella bronchiceptica*	*Citrobacter* spp.	CONs	*Escherichia coli*	*Haemophilus influenzae*	*Klebsiella* spp.	*Moraxella catarrhalis*	Neisseriaceae	Other species of Enterobacteriaceae	Other species of Lactobacillales	*Pseudomonas* spp.	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Streptococcus pyogenes*
R	No. & (%)	1(14.2)	1(33.3)	1(100)	3(75)	1(7.6)	13(81.2)	1(5.2)	4(66.6)	4(100)	2(25)	1(16.6)	11(42.3)	1(14.2)	2(11.7)	4(18.1)	34(54.8)
	Conc.(µg/ml)	5.0	3.5	3.5	3.0	3.0	1.5	4.5	1.5	0.7	3.0	4.0	5.0	4.5	8.0	5.0	12
H	No . & (%)	3(60)	1(100)	-	4(16)	2(6.4)	1(100)..	2(100)	1(16.6)	2(100)	-	1(14.2)	1(4.5)	2(28.5)	18(50)	1(8.3)	8(15.3)
	Conc.(µg/ml)	2.5	0.5		0.7	0.9	5.5	0.05	1.0	0.7		3.5	5.0	2.5	5.5	5.0	2.5

R, isolates of refinery; H, isolates of hospital;CONs,coagulase-negative staphylococci.

4.CONCLUSION

Inhalation of metal particles might be more dangerous than other routes like digestive system or skin. Workers with low experience, associated with short employment, constituted the highest percentage of infections. Bacteria isolated from patients of the refinery were more virulent due to their relative high metal resistance comparing with those of hospital.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

ETHICAL APPROVAL

Ethical clearance for the study was obtained from the Committee of Higher Studies in College of Medicine,University of Tikrit. The researcher did not in any way expose participants of the study to physical or psychological harm. Participation in the study was strictly voluntary with the informed consent of participants that guaranteed their right to privacy. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki."

REFERENCES

1. Chiodo J, Rolfe K. Health effect of eleven hazardous air contaminants and recommended evaluation criteria. Air Quality Technical Report No. 13.2002.
2. Skrtic L. Hydrogen Sulfide, Oil and Gas, and People's Health. M.Sc. thesis.2006.
3. Bruins MR, Kapil S, Oehme FW. Microbial resistance to metals in the environment. *Exocitol Environm Saf.2002;* 45: 198- 207.
4. Ogbo EM, Okhuoya JA. Bioavailability of some heavy metals in crude oil contaminated soils remediated with *Pleurotus tuberregium* Fr. *singer*. *Asian J Biol Sci.2010;* 4: 53-61.
5. Fong ST, Camakaris J, Lee BT. Molecular genetics of a chromosomal locus involved in copper tolerance in *Escherichia coli* K12. *Mol Microbiol.1995;* 15: 1127-1137.
6. Neyt C, Iriarte M, Thi VH, Cornelis GR. Virulence and arsenic resistance in Yersiniae. *J Bacteriol.1997;* 179: 612–619.
7. Stepanauskas R, Glenn TC, Jagoe CH, Tuckfield RC, Lindell AH, King CJ, McArthur JV. Coselection for microbial resistance to metals and antibiotics in freshwater microcosms. *Environm Microbiol.2006;* 8: 1510–1514.

8. Murray RM, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. Manual of clinical microbiology, 7th Ed. . *American Society for Microbiology,Washnigton,DC.;1999.*

9. Mahon CR, Manuselis G, Lehman DC. Textbook of Diagnostic Microbiology, 3rd Ed. USA: Saunders Company.2007.

10. Cruickshank R, Duguid JP, Marmion BP, Swain RH, eds. Medical Microbiology. 12th Ed. New York Churchill Livingstone.1975.

11. Collee JA, Fraser AG, Marmion BP. Practical medical microbiology. New York. Churchill. Livingstone.1996.

12. Forbes BA, Sahm DF, Weissfeld AS. Baily and Scott's Diagnostic Microbiology, 12th Ed. St. Louis, MO: Mosby.2007.

13. Cowan ST, Steel KJ. Cowan and Steel Manual of Medical Bacteriology Cambridge University Press, London.1976.

14. Olukoya DK, Smith SI, Ilori MO. Isolation and Characterization of heavy metals resistant bacteria from Lagos Lagoon. *Folia Microbiol.1997;* 42 (5): 441-445.

15. Archibald L, Phillips L, Monnet D, et al. Antimicrobial resistance in isolates from inpatients and outpatients in the United States: increasing importance of the intensive care unit. *Clin Inf Dis.1997;* 24:211–215.

16. Al-Jebouri,M M, Sharif,A Y and Abdulla,B A (1990) Mercury and antibiotic-resistant *Escherichia coli* from infants stool of Ninevah.Iraqi M J.1990; 38,51-56.

17. Karamalidis,AK,Vondria,EA. Application of stabilization/solidification technology on oil refinery sludge contaminated by heavy metals. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2004;39:961-971.

18. Mark RB, Sanjay K, Frederick WO. *Pseudomonas pickettii*: a common soil and groundwater aerobic bacteria with pathogenic and biodegradation properties. *Environm Res, Sec B, Ecotox Environm Saf.2000;* 47: 105-111.

19. Meargeay M, Monchy S, Vallaeys T, Auquier V, et al. *Ralstonia metallidurans*, a bacterium specifically adapted to toxic metals: towards a catalogue of metal-responsive genes. *FEMS Microbiol Rev.2003;* 27: 385-410.

20. Iwegbue CMA, Williams ES, Isirimah NO. Study of Heavy Metal Distribution in Soils Impacted with Crude Oil in Southern Nigeria Soil and Sediment Contamination. *An International J.2009;*18: 136–143

21. Connell DW, Miller GJ. Chemistry and Ecotoxicology of Pollution.1984; John Wiley& Sons, N. Y. New York.

23.Bada BS,Olarinre,TA. Characteristics of Soils and Heavy Metal Content of Vegetation in Oil Spill Impacted Land in Nigeria. Proceedings of the Annual International Conference on Soils, Sediments, Water and Energy.2012;17:Art 2,1-10.

24.Arnold SR, Straus, SE. Interventions to Improve Antibiotic Prescribing Practices in Ambulatory Care. Evidence-Based Child Health: *A Cochrane Rev J.2006;*1: 691-691.

25.Yilmaz EI. Metal tolerance and biosorption capacity of *Bacillus circulans* strain EB1. *Microbiol.2003;* 154: 409-415.