Heavy Metals Tolerance and Antibiotics Susceptibilty Profiles of *Staphylococcus aureus* Strains Isolated From Clinical Sources in Baquba city

Lina Abdulameer Salman Alsaadi

Department of Biology - College of Science - Diyala University - Iraq

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Abstract

Twenty- one *Staphylococcus aureus* isolated from various clinical samples (wound , ear , pus , urin and burn) collected from Baquba General Hospital over a period (April 2015 to June 2015).Out of 21 *Staphylococcus aureus* isolates , 17 (80.95%) were resistant to methicillin , while 4 (19.04%) were methicillin susceptible. Results of antimicrobial susceptibility showed high resistance to amoxicillin , amoxicillin/clavulanic acid , trimethoprime and cefotaxime with ratio 100% , 76.1% , 76.1% and 71.4% respectively, while isolates resistant oflaxacin with 4.7%. The results showed that 15 isolates of the total (21) of *Staphylococcus aureus* could produce β-lactamase with percentage (71.4%) , 5 isolates produce of Metalloβ-lactamase with (23.80%) , and 4 isolates (19.04%) produce ESβLs. *Staphylococcus aureus* isolates have ability to tolerant the highest concentration of heavy metals like (Cobalt , Copper , iron , Mercury , Zinc) with ratio (57.1% , 52.3% , 66.6% , 33.3% , 61.9%) respectively. Additionally result of plasmid profile presented that all isolates of *Staphylococcus aureus* contained two band s of plasmid vary in size . Plasmid curing was carried out by acridin orang (256µg/ml) , cobalt and zinc resistance character was found to be present on the chromosomal DNA rather than the plasmid DNA whereas iron , copper and mercury resistance characters were found to be present on the

plasmid . Curing result showed the loss of antibiotic and heavy metal resistance property for (cefotaxim , amoxicillin , erythromycin , ciprofloxacin , iron , copper and mercury) from the isolates and confirms a relationship between antibiotic and heavy metal resistance with plasmid.

Key words : *Staphylococcus aureus* , Heavy Metals , Antibiotics , Curing, Plasmid .

نسق التحمل للمعادن الثقيلة والحساسية للمضادات الحيوية لبكتريا *Staphylococcus aureus* المعزولة من مصادر سريرية في مدينة بعقوبة .

لينا عبدالامير سلمان السعدي

العراق- جامعة ديالى - كلية العلوم -قسم علوم الحياة

الخلاصة

جمعت احدى وعشرون عزلة من المكورات العنقودية الذهبية من مصادر سريرية مختلفة شملت (الجروح ، الاذن ، القيح ، الادرار ، الحروق) من مستشفى بعقوبة التعليمي للفترة من نيسان 2015 الى حزيران 2015 . من اصل 21 عزلة اظهرت 17 عزلة (80.95%) مقاومة للمثيسلين بينما 4 عزلات (19.04%) حساسة للمثيسلين . اظهرت نتائج الحساسية للمضادات الحيوية مقاومة عالية لمضادات amoxicillin و amoxicillin/clavulanic acid و trimethoprime و cefotaxime بنسبة 100% ، 76.1% ، 76.1% و 71.4%على التوالي ، بينما كانت مقاومة oflaxacin بنسبة 4.7% . اوضحت النتائج ان 15 عزلة (71.4%) من اصل 21 عزلة تنتج انزيمات البيتالاكتاميز و 5 عزلات قادرة على انتاج انزيمات البيتالاكتاميز المعدنية بنسبة 23.80% و 4 عزلات (19.04%) تنتج انزيمات البيتالاكتاميز واسعة الطيف . اظهرت بكتريا *Staphylococcus aureus* قابلية تحمل اعلى تركيز من المعادن الثقيلة (mercury ، iron ، copper ، cobalt ، zinc) وبنسب 57.1% ، 52.3% ، 66.6% ، 33.3% ، 61.9% على التوالي . بالاضافة الى ذلك اظهرت نتائج النسق البلازميدي احتواء بكتريا *Staphylococcus aureus* على حزمتين بلازميديتين مختلفة الحجم . تمكنت مادة الاكريدين البرتقالي بتركيز(256µg/ml) من تحييد البلازميدات حيث وجد ان صفة المقاومة لمعادن zinc و cobalt يشفر لها الـ DNA الكروموسومي وليس البلازميدي ، بينما صفة المقاومة لمعادن iron ، copper و mercury موجودة على البلازميد . اظهرت نتائج التحييد البلازميدي فقدان معظم العزلات صفة المقاومة للمضادات الحيوية والمعادن الثقيلة (cefotaxim , amoxicillin , erythromycin , ciprofloxacin , iron , copper and mercury) وان ذلك يؤكد العلاقة بين مقاومة المعادن الثقيلة والمضادات مع البلازميدات.

الكلمات المفتاحية : *Staphylococcus aureus* ، المعادن الثقيلة ، المضادات الحيوية ، التحييد ، البلازميدات.

Heavy Metals Tolerance and Antibiotics Susceptibilty Profiles of *Staphylococcus aureus* Strains Isolated From Clinical Sources in Baquba city

Lina Abdulameer Salman Alsaadi

Introduction

Staphylococcus aureus is a Gram-positive bacterium acting as one of the main pathogens that can cause various forms of diseases associated with wound infection , skin infection , soft tissue and more serious sequelae such as urinary tract infection , septicaemia , osteomylitis and endocarditis (1,2). Multidrug resistance is very common in *Staphylococcus aureus* clinical isolates worldwide, particularly in developing countries (3) . Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates have been associated with nosocomial infections and rapidly developed resistance to multiple drug classes and is now regaded as a major hospital acquired pathogen worldwide (4,5) , MRSA not only resistance to methicillin , but it also resistant to many groups of antibiotics, such as ciprofloxacin , co-trimoxazole and gentamicin (6,7) . Heavy metals have been found in increasing proportions in microbial habitates as a result of rapid urbanization and natural processes (8). Metals like copper , nickel , iron , zinc and cobalt play important role both directly or indirectly in almost all metabolic processes , growth and development of microorganisms (9). However increase in the concentration of the metals beyond tolerance levels have forced these microorganisms to adapt using various biological mechanisms in other withstand the condition of the increased concentrations (9,10). the growth inhibition mechanism have been involved the entrance of heavy metals ions (Cu^{+2} , Cd^{+2} , Zn^{+2} , Ag^{+} etc.) leads to the formation of secondary metabolites , subsequently constituting the compounds toxic to the microorganism (11). *S. aureus* was reported to be resistant to anumber of metals such as iron and chromium (12). Many mechanisms enable microbes to adapt with heavy metals presence included : accumulation and uptake , mineralization , complexation , metal efflux systems , reduction of metal ions or utilization of the metal as a terminal electron accepter during an aerobic respiration and metal sorption (13,14).

Microorganisms that can grow and tolerate in the presence of high concentration of heavy metals play an important role in their biological cycling which has great potential in bioremediation of environment contaminated with heavy metals (15). Metal resistance in bacteria is mostly plasmid – encoded (16), the goal of this research is to analyze the antibiotics

sensitivity patterns , tolerance of heavy metals and the relationship between heavy metal and antibiotic resistance with DNA plasmid in *S. aureus* isolates .

Materials and Methods

Media and Chemicals

Mueller-Hinton agar , Nutrient agar , Nutrient broth , Manitol salt agar and Agarose from Oxoid – England , acridine orange , EDTA , cobalt , iron , copper , zinc , Mercury from BDH – England, Antibiotics from Oxoid – England.

Identification of Bacterial Isolates

Twenty- one *Staphylococcus aureus* isolates from various clinical samples (wound , ear , pus , urin , burn) collected from Baquba General Hospital over a period of three months (April 2015 to june 2015) were inoculated into nutrient agar (as slant) and incubated at 37°C for 24 h. *S. aureus* isolates were identified by Gram stain , manitol fermentation , coagulase test , catalase test and colony morphology on manitol salt agar (17).

Antibiotic Susceptibility Testing

Eleven antibiotics including Methicillin (MC) 5µg , Amoxicillin (AX) 25 µg , Amoxicillin-Clavulanic acid (augmentin) (AMC) 30 µg , Cefotaxime (CTX) 30 µg , Pipracillin (PRL) 30 µg , Oflaxacin (OF) 5 µg , Gentamicin (CN)10 µg , Ciproflxacin (CIP) 5 µg , Erythromycin (E) 15 µg , Trimethoprim (TMP) 10µg , and Vancomycin (VA) 30 µg have been tested in order to test the sensitivity of *Staphylococcus aureus* dy using the Mueller-Hinton agar plates . The interprentation of inhibition zones around the disc is according to the guidelines of the National Committee for Clinical Laboratory Standards (CLSI)(18,19).

Detection of β-lactamase , Metalloβ-lactamase and ESβLs Production.

The iodometric method for detection of β-lactamase was described by (WHO) 1978 (20). Detection of Metalloβ-lactamase by using Imipenem EDTA combined disk test (21), using two Imipenem disk (10µg) with 3cm between them , and then the (10µg) EDTA solution to one of the drives of Imipenem then incubated at a temperature 37°C for 18-24 hour, after observing

areas of inhibition zone , increase of inhibition zone above 7mm on the disk Imipenem with EDTA compaired with the Imipenem disk alone , the result is positive . Disk Approximation method was performed for detection of ESβLs for all isolates which were positive to β-lactamase production (22).

Tolerance of Heavy Metals Salts

The two-fold agar dilution susceptibility method is used for determination tolerance of each *Staphylococcus aureus* strains to five metal salts ($CoCl_2.CH_2O$, $FeCl_3$, $CuCl_2.2H_2O$, $ZnCl_2$ and $HgCl_2$) . Different concentration of salts of heavy metals ranged between 0.005mM to 4.5mM of each metal . Resistant strains were always possible to revitalize using pure medium without addition of metal .The plates were incubated at 37°C and observed for growth antimicrobial activity was expressed in terms of zone of inhibition (mm). Each experiment was repeated twice and average was taken (23).

Plasmid Profile (Plasmid DNA analysis)

Plasmid DNA of the four isolates are extracted using the Pure Yield^{TM} Plasmid Miniprep Kit (Promega U.S.A). Plasmid DNA was analyzed by electrophoresis on 0.7% agarose gel containing 0.5µg of ethidium bromide per ml (24). And pass the electricity (7 volt/ cm^2) for (1-1.5) hour , the agarose have been tested by using ultra violete transilluminator in wave length (320nm).

Curing of Plasmid DNA

Curing was conducted by using different concentrations of Acridin orange (64 , 128 , 256 , 512 , 1024 , 2000) µg/ml (24). The tubes were incubated at 37°C for 24h , the cured isolates were checked for their antibiotic sensitivity and heavy metal tolerating capacity.

Results and Discussions

Isolation and Identification of *Staphylococcus aureus*

Twenty- one isolates of *Staphylococcus aureus* were obtained from the total 84 clinical specimens (wound , ear , pus , urin, burn) . Diagnosis was confirmed by using regular api-staph system . Classified all isolates of *S. aureus* by positions of infection (Table 1) , The results showed that the largest proportion of the isolates were within wound samples 7(33.33%) isolates of the total , while the proportion of the isolates in the ear samples 5 (23.80%) isolate , in cases of urin and pus were 4 (19.04%) , wheals the proportion of the isolates in the burns samples were 1 (4.76%). These results have the agreement with Imran *et al* (5) who indicated that the hight rate of infection with *S.aureus* was found in wound samples (25%) , followed by ear swab (20%) , pus swab (18%) and urin (17%) , and also these results are agreed partially with Obiazi *et al* (25) pointed out that the highest rate of infection with *S.aureus* were from infections of wounds , which amounted to (12) case (48%) , followed by cases of infections at the urinary tract as it amounted to (8) isolated (40%) .

Table (1): The number of isolates and ratios as sites of infection .

Type of Clinical Sources	Number of *Staphylococcus aureus* Isolates	Percentage (%) of Isolates
Wound swab	7	33.33%
Ear swab	5	23.80%
Urin	4	19.04%
Pus swab	4	19.04%
Burn swab	1	4.76%
Total	21	100 %

Antimicrobial Susceptibility test

The results of antimicrobial susceptibility test showed that 17 isolates (81%) were found to be resistant to methicillin , while 4 isolates (19%) were sensitive. this percentage agreed with the resuls of El-Gayar *et al* (26) demonstrated the ratio of methicillin resistance in this amounted (81.4%) , while (18.6%) were sensitive.Sensitivity of *S. aureus* aginst (12) antibiotic are tested , which common types in use in our country for treatment of various infection , the results showed all isolates has resistance to amoxicillin with ratio 100% (Figure 1), and these results are consistant with Imran *et al* (5) pointed out that *S.aureus* were resistant to amoxicillin with 100% . *S.aureus* isolates showed resistance to amoxicillin/clavulanic acid and trimethoprime with 76.1% , while *S. aureus* resists cefotaxime , erythromycin , piperacillin with 71.4% , 61.9% , 57.1% respectively . The results showed that *S.aureus* resist Ciprofloxacin with 38% , while resistance of aminoglycoside group including gentamicin was 52.3% . This isolates resists vancomycin and oflaxacin with 23.8% , 4.7% respectively(Figure 1). This resistance may due to change in permeability of the outer membrane , as well as the secretion of β-laclamase enzymes and efflux pump system (27).

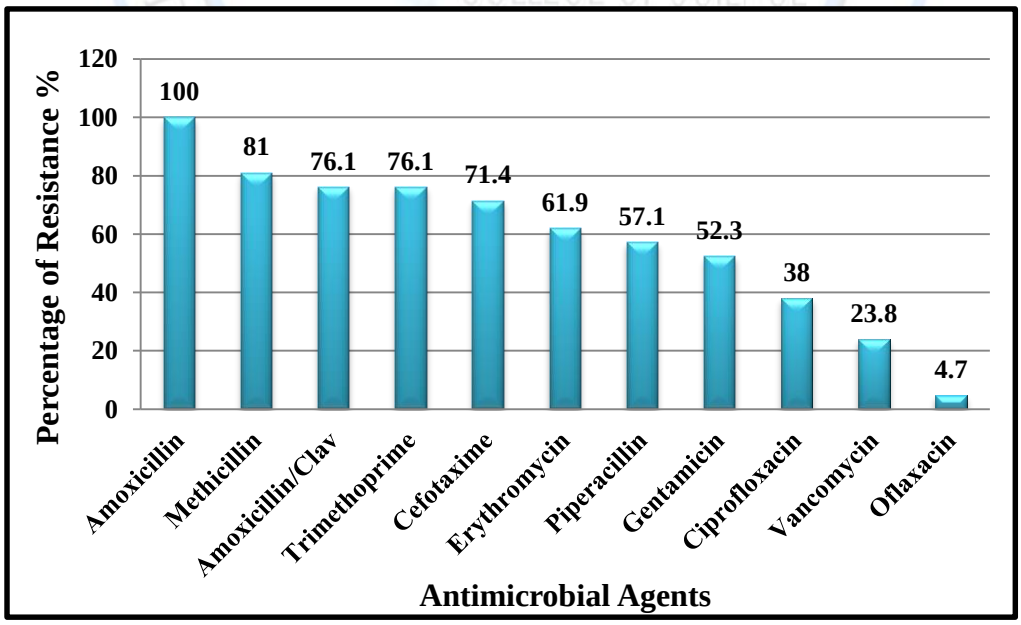

Figure (1) : Antibiotic resistance pattern of *Staphylococcus aureus* .

Detection of β-lactamase , Metalloβ-lactamase and ESβL Production.

The results showed that 15 isolates of the total (21) of *Staphylococcus aureus* gave a positive result for the examination of β-lactamase production with percentage (71.4%) (Table 2) , the results agree with Torimiro *et al* (28) pointed out that the β-lactamase production of *S.aureus* by (70.1%) .The results indicated that 5 (23.80%) isolates were Metalloβ-lactamase producers (Table 2) , While 4(19.04%) isolates of *S.aureus* were ESBLs producers.

Table (2):The percentages of *S.aureus* isolates β-lactamase, Metalloβ-lactamase, ESBLs production.

β-lactamase production	Number of isolates	Ratio %
β-lactamase	15	71.4%
Metalloβ-lactamase	5	23.80%
ESBLs	4	19.04%

***S. aureus* Tolerance to Metal Salts**

The results shown in (Table 3) indicate that 12 isolate (57.1%) of *S.aureus* has the ability to tolerances 1.5mM of cobalt , this is the highest concentration of cobalt metal bacteria able to afford, while 3 isolates have the ability to tolerate low cocentration 0.5mM of metal with percentage (14.2%). As for copper, the highest concentration was 3 mM , *S.aureus* isolates tolerant this metal by 11 isolates (52.3%) , whereas tolerance *S.aureus* lowest concentration 0.5mM by 4 isolates (19%). Copper is associated with particular sites on any amino acid for microorganisms and restores oxidation-reduction cycle and generates free radical hydroxide near the binding sites this causing damage to amino acid (29). 14 isolates (66.6%) of *S.aureus* tolerant a higher concentration of 3 mM of iron , able 3 isolates (14.2%) tolerant a lowest concentration 0.5mM of iron .this results agrees with Singh *et al.* (12) pointed out that all *S.aureus* isolates were resistant to iron salts. The results for mercury demonstrated that (7) *S.aureus* isolates tolerant a higher concentration of 0.03mM of mercury with ratio (33.3%) and

9 isolates tolerant 0.05mM of mercury with ratio (38.5%). The results showed that 13 isolates (61.9%) has ability to tolerant zinc metal with concentration 1.5mM (table 3) , While (19%) of *S.aureus* tolerant lowest concentration 0.5mM of zinc. Samanta *et al* (30) have shown that microorganisms have the capacity to resist antibiotics and heavy metals , which may be extremely harmful to human being and animals. The resistance of bacteria to heavy metals may due to the presence of conjugative plasmid that mediates resistance to metals (31).

Table (3): Percentages of tolerance *S.aureus* isolates for heavy metals .

Heavy metals	Concentration of heavy metals (mM)	N0.&Percentage (%) of *S.aureus* isolates
Cobalt (Co)	1.5 1 0.5	12 (57.1%) 6 (28.5%) 3 (14.2%)
Copper (Cu)	3 2 0.5	11 (52.3%) 6 (28.5%) 4 (19%)
Iron (Fe)	3 1.5 0.5	14 (66.6%) 4 (19%) 3 (14.2%)
Mercury (Hg)	0.03 0.01	7 (33.3%) 9 (38.5%)
Zinc (Zn)	1.5 1 0.5	13 (61.9%) 4 (19%) 4 (19%)

The relationship between the isolates were resistant to heavy metals and antibiotics

S.aureus isolates under study showed different responses to heavy metals and antibiotics ranged between sensitive and resistance to antibiotics . Isolates of *S.aureus* isolated from wound (SW3) has ability to resistant 10 (90.9%) antibiotic and tolerance five heavy metals (Co , Cu , Fe , Hg , Zn) by 100% (Table 4) , while anather isolates isolated from ear (SE8) resistance 5 antibiotics with ratio (45.4%) and tolerance 2 (40%) heavy metal (table 4). The interaction between heavy metals and antibiotic resistance are of three types : heavy metals interaction with antibiotic

resistance genes or even their products, heavy metals interaction with antibiotic compounds and heavy metal interaction with bacterial properties like conjugation (32).

Table (4): Resistance ratios of *S. aureus* to antibiotics and heavy metals.

S.aureus isolates	No. of antibiotic resistance with ratio (%)	No. of heavy metals tolerance with ratio (%)
SW1	8 (72.7%)	5 (100%)
SW2	5 (45.4%)	3 (60%)
SW3	10 (90.9%)	5 (100%)
SW4	7 (63.6%)	4 (80%)
SW5	7 (63.6%)	4 (80%)
SW6	8 (72.7%)	4 (80%)
SW7	9 (81.8%)	5 (100%)
SE8	5 (45.4%)	2 (40%)
SE9	7 (63.6%)	4 (80%)
SE10	9 (81.8%)	5 (100%)
SE11	8 (72.7%)	3 (60%)
SE12	6 (54.5%)	3 (60%)
SU13	9 (81.8%)	4 (80%)
SU14	8 (72.7%)	5 (100%)
SU15	8 (72.7%)	5 (100%)
SU16	6 (54.5%)	4 (80%)
SP17	10 (90.9%)	3 (60%)
SP18	7 (63.6%)	3 (60%)
SP19	6 (54.5%)	3 (60%)
SP20	7 (63.6%)	4 (80%)
SB21	8 (72.7%)	4 (80%)

Plasmid profile of *S.aureus*

Plasmid profile of four isolates has been investigated , especially those that showed tolerant of heavy metals and multiple resistance to antibiotics , the results showed that all isolates of *S.aureus* contained two bands of plasmid vary in size (Figure 2).this results agreed partially with results of Obajuluwa *et al* (33) which found all *S.aureus* isolates containing two bands of plasmid and some isolates contained one mega plasmid .

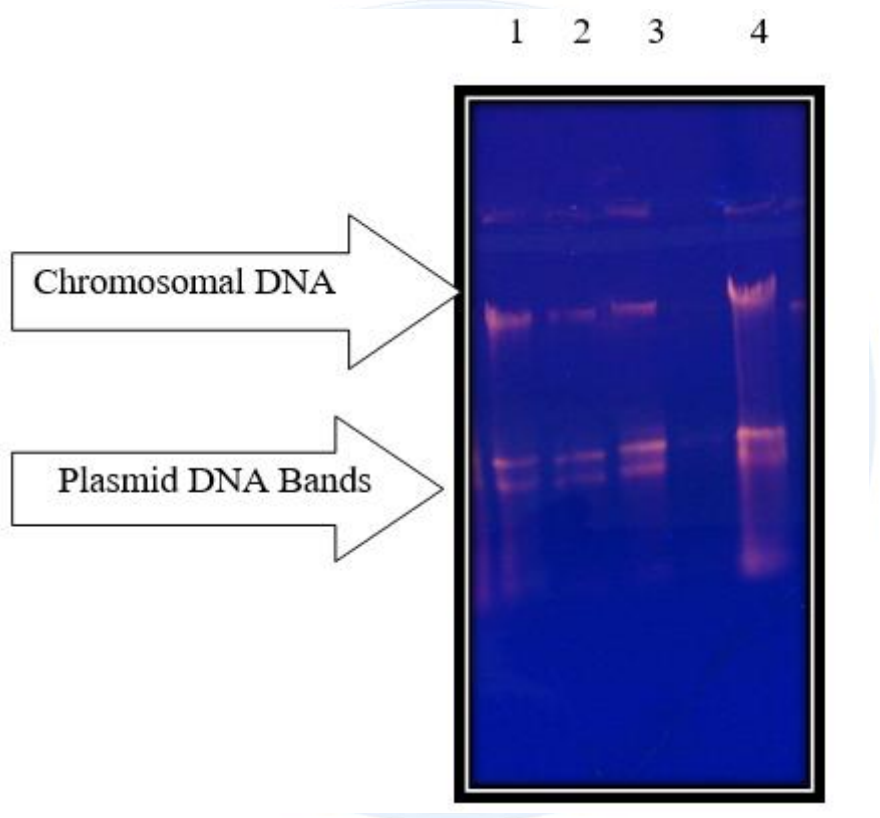

Figure (2): Agarose gel electrophoresis of plasmids from *Staphylococcus aureus*

(1) Plasmid content of SW3 isolate (2) Plasmid content of SW7 isolate

(3)Plasmid content of SP10 isolate (4) Plasmid content of SU15 isolate

Curing of plasmid DNA

Acridin orange was used in order to cure plasmids of *Staphylococcus aureus* isolates . The results showed the best concentration was 256µg/ml , which able to cure plasmids from all isolates . After the curing , the isolates were tested to antibiotic resistance and heavy metal tolerance , all isolates have not loss the ability to tolerate cobalt and zinc this mean that cobalt and zinc resistance gene was found to be present on the cheomosomal DNA rather than the plasmid DNA , while all isolates loss the capacity to tolerate iron , copper and mercury this due to its resistance genes were found to be present on the plasmid .Aditionaly results showed all four isolates lost the capacity to grow on medium containing cefotaxim , amoxicillin , erythromycin , and ciprofloxacin , this indicated that isolates contain genes on their plasmid.

Conclusion

The results suggest the possibility of isolating *Staphylococcus aureus* from different clinical sources . These isolates were resistant to many antibiotics, (used drug of choise) and tolerant of heavy metals . The tolerance of heavy metals and antibiotics was found to be both chromosomal as well as plasmid mediated in the isolates.

References

1. Taylor, A.R. (2013). Methicillin – resistant *Staphylococcus aureus* infections . primary care 40(3):637-654.
2. Zuniga, R. and Nguyen, T. (2013). Skin conditions: emerging drug-resistant skin infections and infestations. FP essentials 407: 17-23.
3. Akerele J and Ahonkhai IA. (2000) . Urinary pathogen and antibacterial susceptibility. A retrospective study of private diagnostic laboratory in Benin City, Nigeria.J.Med. Lab. Sci. 9:47-48.
4. Batabyal , B. ; Kundu, G. and Biswas, S. (2012). Methicillin resistant Staphylococcus. aureus : A Brief Review. International Research Journal of Biological Sciences : 1(7) : 65-71.

5. Imran , M. ; Imran, M. and Khan . S (2015). Multidrug resistance in Staphylococcus aureus isolates from clinical specimens in Northen India. African J. Microbial. Research. 9(51): 2396-2403.

6. Vidhani, S. ; Mendiratta, PL. and Mathur MD. (2001). Study of Methicillin resistant S. aureus from high risk patients. Indian J. Med. Microbial. 19(2):13-16.

7. Chambers HF (2001). The changing epidemiology Staphylococcus aureus . Emerg. Infect. Dis. 7:178-182.

8. Issazadeh, K. ; Jahanpour, N. ; Pourghorbanali, F. ; Raeisi, G. and Faekhondeh, J. (2013). Heavy metals resistance by bacterial strains. Ann. Biol. Res.4(2):60-63.

9. Tamer, A. ; Aysenur ,K. and Sadik , D.(2013). Antibiotic Levels and Heavt Metal Resistance in Gram-Negative Bacteria Isolated from Seawater, Iskenderum Organized Industerial Zone . J. Applied Biological Sciences , 7:10-14.

10. Nikaido, H. (2009). Multidrug Resistance in Bacteria. Annual Review of Biochemstry, 78:119-146.

11. Lim, C.K. ; Hassan, K.A ; Penesyan , A. ; Loper, JE. And Paulsen, I.T.(2013). The effect of zinc limination on the transcriptome of Pseudomonas protegens Pf-5. Enviromental Microbiology. 15(3):702-715.

12. Singh, A. ; Mishra, M. ; Tripathi, P. and Sachan, S.(2015). Resistance of Heavy Metals on some Pathogenic Bacterial Spices. African Journal of Microbiology Research , 9: 1162-1164.

13. Rajbanshi, A. (2008). Study of Heavy Metal Resistant Bacteria in Guheswori Sewage Treatment Plant. Our Nature, 6:52-57.

14. Nageswaran, N. ; Ramteke, PW. ; Verma, OP. and Pande , A (2012). Antibiotic Susceptibility and Heavy Metal Tolerance Pattern of Serratia marcescens Isolated from Soil and Water. J. Bioremediation&Biodegradation , 3, 158.

15. Nyamboya, R.A. ; Okemo, P.O. and Ombori, O. (2013).Heavy Metal and Associated Antibiotic Resistance of Fecal Coliforms , Fecal Streptococci and Pathogens Isolated from Wastewaters of Abattoirs in Nairobi, Kenya. Journal of Applied Biosciences, 64:4858-4866.

16. Nies , D.H. (1992). Resistance To Cadmium , Cobalt , Zinc And Nickel In Microbes." Plasmid 27 (1):17-28.

17. Washington, CW. ; Stephen, DA. ; William, MJ. ; Elmer, WK. ; Paul, CS. ; Gray, WP. and Gail, LW. (2006). Gram positive cocci . Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6t edition. Philadelphia: Lippincott Williams &Williams 12:623-671.

18. CLSI(Clinical and Laboratory Standers Institute)(2010).Performance Standards for Antimicrobial Susceptibility Testing ; 20th Informational supplement, Clinical and Laboratory Standers Institute Wayane, PA: M100-S20:30(1).

19. Shittu, A. ; Lin, J. and Kolawole, DO. (2006). Antimicrobial susceptibility patterns of Staphylococcus aureus and characterization of MRSA in South Western Nigeria . WOUNDS. 18(4): 77-84.

20. Who .(1978). Techniques for the detection of β-Lactamase producing strains of Neisseria gonorrhoae. 616: 137-143.

21. Bhalerao, D.S. ; Roushani, S. ; Kinikar, A.G. and Akhter, I. (2010). Study of Metallo-betalactamase producing Pseudomonas aeruginosa in Pravara Rural Hospital. Pravara Med Rev ;pp 1-5.

22. Jarlier , V. ; Nicolas, M. ; Fournier, G. and Philippon, A. (1988). Extended broud-spectrum β-Lactamases conferring transferable resistance to newer β-Lactam agents in Enterobacteriaceae: Hospital prevalence and susceptibility patterns . Rev. Infect. Dis. 10(4):867-878.

23. Singh , V. ; Chauhan, PK. ; Kanta, R. ; Dhewa, T. and Kumar, V.(2010). Isolation and Characterization of Pseudomonas Resistant to Heavy Metals Contaminations. Int. J. Pharma. Scie. Rev. Res. 3:164-167.

24. Sambrook,J. ; Fritsch , E.F. and Maniatis , T.(1989). Molecular Cloning : A Laboratory Manual. 2nd ed. Cold Spring Harbor , N.Y.

25. Obiazi, H.A. ; Nmorsi, O.P. ; Ekundayo, A.O. and Ukwandu, N.C. (2007). Prevalence and antibiotic susceptibility pattern of Staphylococcus aureus fromclinical isolates grown at 37 and 44°C from Irrua, Nigeria. African J. Micro. Res. pp:057-060.

26. El-Gayar , M.H. ; Aboulwafa, M. ; Aboshanab , K.M. and Hassouna, N.A. (2014). Virulance characters of some methicillin resistant Staphylococcus aureus isolates. MedPub Journals. 5.4:3.3823-3843.

27. Livermore DM. (2002). Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa: our worst nighmare? Clinical Infectious Diseases, 34:634-640.

28. Torimiro , N. ; Moshood , AA. And Eyiolawi , SA.(2013). Analysis of Beta-lactamase production and Antibiotics resistance in Staphylococcus aureus strains. J. Infe. Dise&Immu.5(3):24-28.

29. Borkow , G. and Gabbay, J.(2005). Copper as a biocidal tool. Current Medicinal Chemistry , 12:2163-2175.

30. Samanta, A. ; Bera, P. ; Khatun, M. ; Sinha, C. ; Pal, P. ; Lalee, A. and Mandal, A. (2012). An investigation on heavy metal tolerance and antibiotic resistance properties of bacterial strain Bacillus sp.isolated from municipal waste. J. Microbiol. Biotechnol.Res. 2(1):178-189.

31. Egbeb , A.O. and Famurewa, O.(2011). Heavy Metal Resistance among Klebsiella Isolates in some parts of Southwest , Nigeria. Asi.J.of Medical Sciences 3(5):183-185.

32. Nishino , K. ; Nikaido, E. and Yamaguchi, A. (2007). Regulation of multidrug efflux systems involved in multidrug and metal resistance of Salmonella enteric serovar Typhimurium. Journal of Bacteriology .189: 9066-9075.

33. Obajuluwa, A.F. ; Olayinka, B.O. ; Adeshina, G.O. ; Onaolapo, J.A. and Afolabi-Balogun, N.B. (2015). Plasmid profile of Staphylococcus aureus from orthopaedic patients in Ahmadu Bello University Teaching Hospital Zaria, Nigeria.African. J. Micro.Research. 9(33):1941-1946.