Antibiotic susceptibility profile of urinary tract isolates of *Staphylococcus aureus*

Adeleke O E* [1] and Olarinde J D [2]

[1&2]Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria
adelzek@yahoo.com

Abstract: *Staphylococcus aureus* has a common occurrence in the urinary tract infections thereby necessitating a regular monitoring of the susceptibility of organism to antibiotics. Fifty isolates of *S. aureus* from urine samples of patients attending University College Hospital, Ibadan were collected and screened by standard biochemical and microbiological procedures. Antibiotic susceptibility of the isolates was carried out by disk-diffusion method; their ability to produce beta-lactamase was tested by the cell suspension iodometric method. The minimum inhibitory and minimum bactericidal concentrations (MIC and MBC) of augmentin, ceftriaxone and penicillin G against twenty of the isolates were determined by the tube-dilution technique. All the isolates investigated were *Staphylococcus aureus,* and 84% of them produced beta-lactamase. The *S. aureus* strains were highly sensitive to nitrofurantoin (78%), ofloxacin (70%) and augmentin (66%) but showed high resistance to cotrimoxazole (80%), tetracycline (60%), nalidixic acid (78%), amoxicillin (78%), gentamicin (60%) and novobiocin (44%). Over 70% of all the isolates exhibited multiple antibiotics resistance. Sixty percent of the twenty (12) isolates whose MIC and MBC were determined were sensitive to ceftriaxone while only 25% (5) were sensitive to augmentin. All the isolates were resistant to penicillin G. *S. aureus* remains a common aetiology of urinary tract infection among patients and the drugs of choice could be nitrofurantoin, ofloxacin, augmentin and ceftriaxone. The high multiple antibiotic resistance observed underscores the need to conduct antibiotic susceptibility testing before prescription in order to avoid therapeutic failure.

1. Introduction

Urinary tract infection (UTI) is a notorious problem in both the community and hospital practices, affecting people of all ages and gender. The infection presenting itself in many patients with clinical syndromes such as cystitis, pyelonephritis and sometimes prostatitis, the inflammations of the bladder, kidney and prostrate respectively, causes them to visit their physicians annually. The short urethra in females is a less effective deterrent to invasion by uropathogens; and except at extremes of life, the infection affects more females than males, with at least one-fifth of them infected at a point during their lifetime, though most infections in women are asymptomatic (Mims *et al*., 1998, Mullenix and Prince 1999).

Primarily, organisms gain access to the urinary tract via the urethra, or less commonly, the descending route through the blood. The most frequent causes of the infection are bacterial species of the family Enterobacteriaceae and *Escherichia coli* is usually the most common in both in-patients and out-patients (Mullenix and Prince 1999). Other Gram-negative bacteria are *Pseudomonas aeruginosa* and *Acinetobacter* spp. Gram-positive bacteria including *Enterococcus faecalis*, capnophilic corynebacteria and lactobacilli, and staphylococci, which used to play minimal roles, have now assumed significant places as etiological agents of UTI (Mims *et al*., 1998, Mbata, 2007).

The genus *Staphylococcus* comprises over forty species and majority of them are saprophytic or rare pathogens. *S. epidermidis*, *S. haemolyticus* and *S. saprophyticus* are coagulase-negative and frequently cause UTI in humans (Cunha *et al*., 2004). The increased use of invasive procedures in debilitated patients led to their emergence in recent times. *S. aureus* is a coagulase-positive opportunistic pathogen known to cause series of pyogenic infections and intoxications in immunocompetent and immunocompromised individuals (Odugbemi and Coker 1987). It is also implicated in ascending urinary tract colonization and infection, sometimes leading other bacteria including *E. coli* (Abdul and Online, 2001; Akinloye *et al*., 2006). Omoregie *et al (*2008) reported *S. aureus* to be the most common agent of the infection in both diabetic and non-diabetic patients that attended a tertiary hospital in Benin, Nigeria. Its prevalence is however low in the general population but rises in patients undergoing urologic procedures. *S. aureus* bacteriuria used to be dismissed as colonization since most patients do not show the classical symptoms of UTIs (Muder *et al.,* 2006).

Despite the availability of different antibiotics for managing UTI, patients are refractory to permanent cure. Prolonged freedom from the infection

is found in as few as 10% of the patients (Kass, 1955). Development of resistance to antibiotics is a common phenomenon in uropathogenic bacteria, especially *S. aureus*. For instance, penicillin, introduced into clinical use in1940s which was highly effective against staphylococci, had become of less clinical value by the late 50s because resistant variants of staphylococci had emerged and were widespread worldwide (Kateřina, 2004). Most strains of *S. aureus* possess the ability to produce beta-lactamase, an enzyme that can open beta-lactam rings in cephalosporins and penicillins. Some acquire resistance genes from the environments and /or from other bacteria and thus may exhibit resistance to antibiotics in other classes. Typical of these are the methicillin-resistant *S. aureus* (MRSA) strains that bear the *mec* gene.

The increasing bacterial resistance to commonly available drugs is of great concern to physicians as it continues to limit the therapeutic option available to patients. Physicians depend on the result of susceptibility tests instead of empirical prescription to properly treating the infection. The suggestion given in Ciba foundation symposium in 1996 about controlling mounting antibiotic resistance includes accurate identification of species causing infection and the regular conduct and monitoring of their antibiotic susceptibility (Singlet, 1997). Antibiotic susceptibility pattern of uropathogens varies over time and from one geographical location to another. There is the need to update data on susceptibility patterns of bacteria, hence, the need for the study being reported.

2. Materials and Methods

Bacterial strains

Fifty staphylococcal isolates from midstream urine sample of patients were obtained from the Department of Medical Microbiology University College Hospital, Ibadan between October 2008 and July 2009. The isolates were Gram-stained and screened on different media including: blood agar, mannitol salt agar and DNase test agar. Production of coagulase by the tube method, catalase activity, oxidase test, growth in thioglycollate broth, growth in 15% Sodium chloride, acid production from glycerol in the presence of 4μg/ml erythromycin and oxidation-fermentation tests using different sugars: xylose, mannose, maltose, glucose and trehalose were carried out as described by Cheesebrough, 2000; MRS Cunha *et al.*, 2004.

Antimicrobial susceptibility testing

The Kirby-Bauer agar diffusion method (Cheesebrough, 2000) was used to test each of the strains and control strain, *S. aureus* ATCC25923 against the following antibiotics: amoxicillin (25μg), co-trimoxazole (25μg), nalidixic acid (30 μg), gentamicin (10μg), ofloxacin (30μg), augmentin (30μg), tetracycline (30 μg) (Abtek Biological Limited) and novobiocin 5 μg (Oxoid Ltd). Overnight broth culture of each isolate adjusted to match 0.5 McFarland standard (10^8 CFU/ml) was used to seed well dried Mueller-Hinton agar plates and each plate was incubated at 37°C for 24 h. Zones of growth Inhibition measured were scored as resistance, intermediate or sensitive according to the interpretation standard of NCCLS (1999)chart.

Multiple antibiotic resistance (MAR) index was calculated as follows:

MAR index for isolates = number of antibiotics to which the isolate is resistant/number of antibiotic tested.

Detection of beta-lactamase

Cell-suspension iodometric method as described by Sykes (1978) was used to detect beta-lactamase production in each of the bacterial isolates. Briefly, cell suspension of density 10^9cells/ml was homogenized in 0.5 ml phosphate buffered solution containing penicillin G (10000 units or 0.06mg/ml) and left to stand for 1h before two drops of freshly prepared 1% aqueous soluble starch were added. The mixture was shaken gently after which a drop of iodine was added. A change of colour of mixture from blue/blue–black to colourless within 10minutes was positive for beta-lactamase production.

Determination of minimum inhibitory concentration (MIC)

MICs of ceftriaxone, augmentin and penicillin G were determined for twenty selected isolates by tube broth-dilution method. Two high concentrations (80 and 100 μg/ml) of each antibiotic prepared in double strength nutrient broth were serially diluted double-fold in decreasing strength. To each dilution was added 0.1ml of 10^{-2} diluted overnight culture of the isolates. All the tubes were incubated at 37°C for 24 h. Control strain was included in the test-run.

Determination of minimum bactericidal concentration

Mixture in the tubes that showed no visible growth in the MIC test was streaked on drug-free nutrient agar plates. The plates were incubated at 37°C for 24 h and the lowest concentration for which no growth was seen on the plate was taken as the MBC.

Statistical analysis was by Chi-square and the value of $p < 0.05$ was deemed statistically significant.

3. Results

All the isolates were Gram-positive cocci observed as cluster, pairs and tetrads. They were all catalase -positive but negative for oxidase activities. The result for distinguishing staphylococci from micrococci and other genera showed that all the isolates fermented glucose under both aerobic and anaerobic conditions, and grew in broth containing 15% sodium chloride. 94% of all the isolates

produced acid from medium containing glycerol-erythromycin (4μg/ml).

Further tests on the isolates revealed that they were all coagulase-positive on human plasma. All the staphylococci were also positive for DNAse activity. Of all the staphylococcal isolates, 94% fermented sucrose, maltose and trehalose, while 98% fermented mannose and 34% fermented xylose. Under anaerobic condition 94% of the isolates fermented mannitol while only 86% produced acid from mannitol aerobically. All the isolates grew in thioglycollate broth. 40% of all the isolates showed beta-haemolysis. All the fifty strains investigated were *Staphylococcus aureus*.

In susceptibility test, a high proportion of the staphylococcal isolates were sensitive to nitrofurantoin, ofloxacin and augmentin. The strains were moderately sensitive to novobiocin but highly resistant to co-trimoxazole, nalidixic acid and tetracycline. More than seventy percent of the strains exhibited multiple antibiotic resistances and majority of the isolates (38%) were resistant to five of the antibiotics tested (Tables 1 and 2).

Table 3 shows the pattern of antibiotic sensitivity of beta-lactamase producers and non beta-lactamase producers. Eighty-four percent of all the strains produced beta-lactamase, out of which 83.33, 78.57, 76.19, 71.43 and 59.52% were resistant to co-trimoxazole, nalidixic acid, amoxicillin, tetracycline and gentamicin respectively.

The minimum inhibitory concentration obtained for augmentin against twenty of the isolates ranged from 0.3-100μg/ml while both ceftriaxone and penicillin G had a similar range of 2.5-100μg/ml. All antibiotic tested had same MBC range of 2.5-100μg/ml. Twenty-five of the isolates were sensitive to augmentin and the minimum inhibition

concentration that inhibited 50% of all the isolates MIC_{50} was ≤100μg/ml. Sixty percent of the isolates were sensitive to ceftriaxone and MIC_{50} was ≤ 20μg/ml. Most of the strains sensitive to the two antibiotics had MBCs that were twice as high as their MIC. One isolate showed high sensitivity to augmentin with its MIC much lower than its MBC (c.a 30 times) (Tables 4 and 5).

Table 1. Antibiogram of *S. aureus* isolated from urinary tract infection cases**.**

Antibiotics	Sensitive	Intermediate	Resistance
Novobiocin[a]	26(52%)	2(4%)	22(44%)
Amoxicillin	11(22)	0(0)	39(78)
Cotrimoxazole	8(16)	2(4)	40(80)
Nitrofurantoin	39(78)	6(12)	5(10)
Gentamicin	17(34)	3(6)	30(60)
Nalidixic acid	8(16)	3(6)	39(78)
Ofloxacin	35(70)	5(10)	10(20)
Augmentin	33(66)	7(14)	10(20)
Tetracycline	14(28)	0(0)	36(72)

[b]Novobiocin(5 μg) not included in NCCLS, defined by Koneman *et al.,* 1997

Table 2. Multiple Antibiotic Resistance (MAR) Index of the isolated *S. aureus* in Ibadan, Nigeria

MAR index	Frequency of MAR Index (n=50) (100%)
0.00	1(2%)
0.11	4(8)
0.22	3(6)
0.33	5(10)
0.44	1(2)
0.55	19(38)
0.66	9(18)
0.77	5(10)
0.88	0(0)
1.00	3(6)

Table 3. Antibiotic sensitivities of beta-lactamase -producing (BLP) and Non beta-lactamase- producing (NBLP*) S. aureus* isolates (%)

Antibiotics	BLP n=42 (84%)				NBLP n=8 (16%)			X^2
	Sensitive	Intermediate	Resistance		Sensitive	Intermediate	Resistance	
Novobiocin	22(52.38%)	2(4.76%)	18(42.86%)		4(50.00%)	0(0.00%)	4(50%)	0.07
Amoxicillin	9(21.43)	0(0.00)	33(78.57)		2(25.00)	0(0.00)	6(75.00)	0.04
Co-trimoxazole	5(11.90)	2(4.76)	35(83.33)		3(37.50)	0(0.00)	5(62.50)	3.02
Nitrofurantoin	32(76.19)	5(11.90)	5(11.90)		7(87.50)	1(13.50)	0(0.00)	1.06
Gentamicin	7(16.67)	3(7.14)	32(76.19)		1(13.50)	0(0.00)	7(87.50)	0.08
Nalidixic acid	14(33.33)	3(7.14)	25(59.52)		3(37.50)	0(0.00)	5(62.50)	0.14
Ofloxacin	29(69.05)	5(11.90)	8(19.05)		6(75)	0(0.00)	2(25.00)	0.04
Augmentin	25(59.52)	7(16.67)	10(23.08)		8(100)	0(0.00)	0(0.00)	2.98
Tetracycline	12(28.57)	0(0.00)	30(71.43)		2(25.00)	0(0.00)	6(75.00)	0.04

Table 4. Minimum inhibitory concentrations and minimum bactericidal concentrations of Penicillin G, Augmentin and Ceftriaxone against strains of *S. aureus*

	BLP	Penicillin G			Augmentin			Ceftriaxone	
		MIC	MBC		MIC	MBC		MIC	MBC
Strain code		(µg/ml)			(µg/ml)			(µg/ml)	
SA01	-	>100	>100		>100	>100		10	20
SA02	-	>100	>100		5	10		5	10
SA03	-	>100	>100		10	10		2.5	5
SA04	+	>100	>100		1.2	10		2.5	5
SA05	-	40	100		20	20		20	20
SA06	+	20	>100		0.3	10		10	20
SA07	+	50	>100		40	80		>100	>100
SA08	-	>100	>100		>100	>100		20	100
SA09	+	2.5	5		>100	>100		>100	>100
SA10	+	>100	>100		>100	>100		>100	>100
SA11	+	>100	>100		>100	>100		5	80
SA12	+	>100	>100		>100	>100		80	100
SA13	+	2.5	5		2.5	5		20	40
SA14	+	>100	>100		5	10		40	40
SA15	+	2.5	25		25	>100		10	20
SA16	+	>100	>100		>100	>100		>100	>100
SA17	+	>100	>100		100	100		>100	>100
SA18	+	>100	>100		>100	>100		>100	>100
SA 19	+	>100	>100		100	100		>100	>100
SA20	+	>100	>100		100	100		50	100
ATCC25923	+	>100	>100		>100	>100		80	100

Table 5. Summary of Resistance pattern of Twenty Staphylococcal strains

Antibiotics	Resistance rate %
Penicillin	100
Augmentin	75
Ceftriaxone*	40

4. Discussion

The high predictive values for the various methods used in identifying staphylococci confirm the fact that they can be used in rapid differentiation of staphylococci from micrococci. Both tube coagulase and DNAse tests have shown that all the bacterial isolates are *S. aureus*. Coagulase test is often used in rapid blood cultures to identify *S. aureus* to a great advantage over the latter (Ajuwape and Aregbesola, 2001). The result of the fermentation test also conforms to the findings of other workers (Ajuwape and Aregbesola, 2001). It however does not agree that all strains of *S. aureus* must completely ferment mannitol, sucrose, maltose and mannose. The incidence of beta-haemolysis in this study is higher (45.7%) than incidence in coagulase-positive *S. aureus* as previously reported by Ajuwape and Aregbesola (2001). Haemolysin production, however, is not a good index for distinguishing *S. aureus* and coagulase–negative staphylococci (Bello and Qahtani, 2005).

S. aureus is a major pathogen associated with many pus infections such as boils, abscesses; and urinary tract infections. It is widely spread due to its non fastidiousness, its hardiness and ability to survive long in susceptibility hosts. It is the most frequent organism isolated from cultures (Akerele and Akonkhai, 2000). The organism was the leading agent of UTI among patients suffering from the infection (Abdul and Online, 2001; Akortha and Ibadin, 2008). Adeleke *et al.* (2002) also found *S. aureus* more prevalent than the coagulase-negative *S. saprophyticus* from urine culture. *S. saprophyticus* is reputed to be an important cause of urinary tract infection among sexually active female adolescents.

More than 70% of all the isolates have MAR index greater than 0.2. This implies that majority of the isolates have been exposed to several antibiotics (Ehinmidu, 2003). The high resistance rates exhibited by the strains of *S. aureus* investigated against amoxicillin (78%), cotrimoxazole (80%), nalidixic acid (78%) and tetracycline(72%) clearly show that these commonly available and cheap drugs are not useful in empirical treatment of UTI except as indicated by the result of antibiotic susceptibility testing. The result of the study supports the similar marked resistance of *S. aureus* to cotrimoxazole, nalidixic acid and tetracycline noticed by Akortha and Ibadin (2008).

Similarly, Ehinmidu (2003) observed a very high resistance rate of *S. aureus* against tetracycline and amoxicillin in Zaria, Nigeria. In this study, the strains of *S. aureus* also exhibited high resistance to gentamicin, parenteral product. The result is not consistent with earlier reports by both Shittu and Mandere (1999) and Ehinmidu (2003) in Zaria that indicated high sensitivity of *S. aureus* to the antibiotic. Good in-vitro activity of gentamicin against the bacterial isolate was noticed by Mezue *et al.* (2006) working in Nsukka. These differences in sensitivity pattern of *S.aureus* could be attributed to abuse and misuse of the antibiotic by patients who certainly are aided by healthcare givers because of the peculiar route of administration of the drug.

The fluoroquinolones, represented in this study by ofloxacin, are no doubt a reliable therapeutic intervention in UTI because they exhibit broad-spectrum activity. *S. aureus* did not show high susceptibility compared to previous reports (Odelola and Idowu 2007).

Expression of beta-lactamase is the most common mechanism of bacterial resistance to beta-lactams (Ahmed, 1996; Smith, 2004). In this study *S. aureus* showed 84% rates of beta-lactamase production. A similar and close figure of 78% for the production of beta-lactamase was obtained for *S. aureus* isolated from postoperative wound swabs (Ahmed *et al.*, 1996). Although, over ninety percent of beta-lactamase *S. aureus* were resistant to amoxicillin; there is no significant association between beta-lactamase production and resistance of *S. aureus* to amoxicillin. Also there is no association between beta-lactamase production and resistance of *S. aureus* to other antibiotics tested except ceftriaxone. Seventy–five percent of amoxicillin resistant strains were non beta-lactamase producers, indicating that other mechanisms of resistance may play a major role as well

Ceftriaxone is one of the third-generation cephalosporins that were introduced to treat infection caused by penicillin-resistant staphylococci because of their stability to the degradative property of beta-lactamase that make most beta-lactams less effective agents. The results from this study clearly indicate that ceftriaxone had better activity than augmentin and penicillin G. It is consistent with the findings of Abd-Elalim and Khalaf (1988), who had reported that both ceftriaxone and ceftazidime were most active against 90% of the bacterial isolates tested including *S. aureus* studied by agar-dilution method. Augmentin had the lowest activity among the three agents.

S. aureus is often considered to be sensitive to novobiocin, an antibiotic used to distinguish *S. epidermidis* and *S. saprophyticus*. This study has demonstrated otherwise. Isolates of *S. aureus* showed moderate sensitivity to novobiocin. This is surprising since it is not used in treating staphylococcal infections in humans because staphylococci develop resistance to it easily. Its use is limited to veterinary practice and diagnostic laboratory.

In conclusion, *S. aureus* was the only species identified with cases of UTI among the patients in the study. The effective drugs for the bacterial isolates are nitrofurantoin, augmentin, ceftriaxone and ofloxacin. This indicates limited alternative in the range of available antibiotics that can be used in the treatment of UTI caused by Gram-positive *S. aureus* due to massive antibiotic resistance.

Corresponding Author:
Adeleke OE
Department of Pharmaceutical Microbiology,
University of Ibadan, P.O Box 22039, Orita U.I Post office, Ibadan, Nigeria.
E-mail: adelzek@yahoo.com
Tel: 08023896439

References
1. Abd-Elalim EAT, Khalaf RM. Comparative in vitro activity of amoxicillin/clavulanate, ceftazidime and ceftriaxone against hospital strains of Gram-positive and Gram-negative bacteria. Chemoterapia1988; 7(2):75-78.
2. Abdul F, Online AB. Bacterial isolates from the urine of women in Ilorin and their antibiotic susceptibility patterns Journal of Obstetrics and Gynaecology 2001;18:16.
3. Adeleke OE, Oluwole FA, Lele A, Esso A. *Staphylococcus aureus* and *Staphylococcus saprophyticus* in urinary tract infections: comparative prevalence and antimicrobial sensitivity. African Journal of Medical and Pharmaceutical Science 2002; *6*:1-5.
4. Ahmed EA, Ahmed EM, Yousif MA, Arabi YE. The relationship between bacterial beta-lactamase production and antibiotic resistance in Khartoum. African Journal of Medicine and Medical Sciences 1996; 25:133-136.
5. Ajuwape ATP, Aregbesola EA. Biochemical characterization of staphylococci isolated from rabbits. Israel Journal of Veterinary Medicine 2001;56(2):1-5.
6. Akerele J, Ahonkhai IA.. Urinary pathogen and antibacterial susceptibility. A retrospective

study of private diagnostic laboratory in Benin City Nigeria. Journal of Medical. Laboratory Science 2000; 9: 47-48.

7. Akinloye O, Ogbolu DO Akinloye OM, Terry Alli OA. Asymptomatic bacteriuria of pregnancy in Ibadan Nigeria: a re-assessment. British Journal of Biomedical Science 2006; 63:109-112.

8. Akortha EE and Ibadin OK. Incidence and antibiotic susceptibility pattern of *Staphylococcus aureus* amongst patients with urinary tract infection in Benin City Nigeria. African Journal of Biotechnology. 2008; 7(11):1637-1640

9. Bello C.S.S, Qantani A. Pitfalls in the routine diagnosis of *Staphylococcus aureus*. African Journal of Biotechnology 2005; 4(1):83-86.

10. Cheesebrough M. 2000. District laboratory practice in tropical countries Part 2. UK: Cambridge University Press.pp134-143

11. Cunha MRS, Sinzato YK, SilveiraL,VA. Comparison of methods for the identification of coagulase-negative Staphylococci. Memorias do Intsituto Oswaldo Cruz 2004; 99 (8):855-860.

12. Ehinmidu JO. Antibiotic susceptibility patterns of urine isolates in Zaria Nigeria. Tropical Journal of Pharmaceutical Research2003; 2:223-228.

13. Idowu OA, Odelola HA. Prevalence of some uropathogenic bacterial isolates and their susceptibility to some quinolones. African Journal of Biomedical Research 2007; 10:269-273.

14. Kateřina B. 2004. Review of antibiotics. Retrieved August 3, 2012, from http://http://old.lf3.cuni.cz/studium/materialy/infekce/en_atb.pdf

15. Koneman EW Allen SD Janda WM Schreckenberger PC, Winn Jr WC. *Color atlas and textbook of diagnostic microbiology*. 5th ed. Philadelphia: Lippincott, 1997; pp 1395.

16. Mbata T. Prevalence and antibiogram of urinary tract infections among prison inmates In Nigeria. The Internet Journal of Microbiology. 2007; 3:2.

17. Mezue K Ofong C Nmezi D, Ugochukwu-Obi G. Antibiotic sensitivity patterns in urinary tract infections at a tertiary hospital. University of Nigeria Medical Students Association 2006

18. Mimms C, Playfair J, Riott I, Wakelin D, Williams R.. Urinary tract infection. *Medical Microbiology*. 2nd ed. London: Mosby International Ltd. 1998; 18:221-228.

19. Muder RR, Brennen C, Rihs JD, Wagener MM, Obman A, Stout JE, Yu VL Isolation of Staphylococcus aureus from the urinary tract: association of isolation with symptomatic urinary tract infection and subsequent staphylococcal bacteremia. Clinical Infectious Disease. 2006 ; 42(1):46-50

20. Mullenix TA, Prince RA.. Urinary tract infections and prostatitis. *Pharmacotherapy: a pathophysiologic approach*. Eds. J.T. DiPiro, RL. Talbert G.C. Yee *et al.;* 4th ed. Connecticut: Appleton & Lange 1999; pp 1779-1794.

21. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing; tenth informational supplement vol. 18 no. 1. National Committee for Clinical Laboratory Standards Wayne Pa, 1999

22. Odugbemi TO, Coker AO. Prevalence of hospital-acquired infection: cure and control. Journal of Infectious Diseases 1987; *4*(6):15.

23. Omoregie R, Erebor JO, Isibor JO, Ogefere HO. Observed changes in the prevalence of uropathogens in Benin City Nigeria. Journal of Medical Laboratory Science 2008;29-31

24. Shittu SO, Mandere MU. Asymptomatic bacteriuruia in antenatal patients: in A.U.T.H Zaria. *Tropical* Journal of Obstetrics and Gynaecology 1999; 16(1):41.

25. Singleton P. Man against bacteria. *Bacteria in Biology Biotechnology and Medicine*. 4th ed. John Wiley and Sons Publisher. 1999; 331-337.

26. Smith A. Bacterial resistance to antibiotics. *Hugo and Russell's Pharmaceutical Microbiology*. Eds. SP. Denyer, NA. Hodges and SP. Gorman.7th ed. Oxford: Blackwell Science. 2004; pp 220-232.

27. Sykes RB. Methods for detecting beta-lactamases. *Laboratory methods in antimicrobial therapy*. Eds. D.S. Reeves, I. Phillip, JD, Williams and R. Wise. Edinburgh: Churchill Livingstone. 1978; pp 44-75.

12/12/2013