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Plasmid profile of multi antibiotic resistant *staphylococcus aureus* isolated from diabetic wounds from patients at Nsukka, South-eastern, Nigeria

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Multi-drug resistant bacterial strains evolving worldwide has created a great public health problem that needs urgent attention; as such bacteria show resistance to the drug of choice for treatment as well as being resistant to newer and last line antibiotics. In this study, the antibiotic susceptibility, multi antibiotic resistance (MAR) index and plasmid profile of MDR *Staphylococcus aureus* isolated from diabetic wounds of patients presenting at Renaissance hospital Nsukka, Southeast, Nigeria were investigated. Using basic bacteriological and biochemical techniques, *S. aureus* was isolated from all 34 specimens and 19 of these showed multi-drug resistances to most of the commonly prescribed antibiotics in the region, with methicillin and vancomycin inclusive. The 19 MDR isolates were screened for the presence of plasmids as well as calculating the multi-antibiotic resistance (MAR) index. The results show the presence of plasmids in 18 (94.73%) of the specimens; while there was no plasmid in one. The plasmids varied in the range of their molecular sizes and nine different plasmid profile groups were identified ranging between 4946 (bp) to 12130 (bp). For the 19 MDR isolates, the calculated MAR index was greater than 0.2. The findings from this study show that 56% of the isolated *S. aureus* were not susceptible to current antibiotics. This could suggest an imminent change in resistant pattern as observed, particularly in an area already reported as high antibiotic use.

Key word: *Staphylococcus aureus,* susceptibility, antibiotic resistance, plasmids, diabetic wound.

INTRODUCTION

The challenge of multidrug resistant (MDR) bacterial strains is enormous, particularly in this 21st century. With the threat posed by MDR bacteria, there should be an urgent need to curtail their menace and reverse their imminent spread. Recently, methicillin resistant *Staphylococcus aureus* (MRSA) has emerged worldwide

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as an endemic pathogen. Incidence of MRSA is on the increase globally and at an alarming rate (Mishra et al., 2013). The bacterium is also reported to be resistant to many new drugs as well (Gould et al., 2012). MRSA is the cause of a number of human infections, some of which are fatal, invasive and could lead to toxic conditions (Subedi and Brahmadathan, 2005; Dhanoa et al., 2012). The fact that *S. aureus* infections are found to be prevalent in hospitals, the community has highlighted the need for an immediate action according to Brennan et al. (2012). MRSA is reported to have distinctive phenotypic and genetic features whether community or hospital acquired (Mammina et al., 2012). Evidence have shown that some strains of *S. aureus* are found to be resistant to the last line antibiotics (Park and Lui, 2012; De Vriese and Vandecasteele, 2014) and this has restricted the options available for its treatment in many regions of the world (Subedi and Brahmadathan, 2005). One of such regions is Nigeria (Akinyemi et al., 1997) where antibiotic resistance may be due to spontaneous mutation or acquired through plasmid transfer from other resistant bacteria (Bhaktar et al., 2003). Therefore, in many instances, resistance to antimicrobial agents by Staphylococci is attributed to the presence of plasmids that carry the genetic determinants for resistance (Adeleke et al., 2002). However, other workers have attributed this behaviour to chromosomal mutation (Chibuike et al., 2014). Plasmids have the ability to mediate the production of drug inactivating enzyme such as β lactamases (King et al., 2006; Diep et al., 2008; Esimone et al., 2010). Reports have indicated that plasmid-encoded antibiotic resistance encompasses most classes of antibiotics currently in clinical use (Esimone et al., 2010). Studies have also shown that plasmid profiles are useful in epidemiological surveillance of disease outbreaks and in tracing antibiotic resistance (King et al., 2006; Diep et al., 2008). Different patterns of antibiotic resistance and plasmid profiles among strains of *S. aureus* have been reported using plasmid profile (Bhaktar et al., 2003; Diep et al., 2008). In the present study, we report the isolation of multi-antibiotic resistant *S. aureus* from diabetic wounds of out-patients attending Renaissance Hospital at Nsukka, Nigeria. Their pattern of antibiotic susceptibility was determined in addition to multi antibiotic resistance (MAR) index and plasmid profile.

MATERIALS AND METHODS

Bacteria strain

Multi-antibiotic resistant *S. aureus* isolated from wounds of diabetic outpatients attending Renaissance hospital, Nsukka, Enugu State of Southeastern Nigeria were used for this study. A criterion for inclusion in this study was to sample patients who have had these wounds for three months and above; indication that wounds are not healing despite treatment. Informed and written consent were obtained from patients before the start of sampling with the permission of the Renaissance hospital, after the study protocol was approved by the departmental ethical committee. The study protocol was in accordance with good clinical practice and ethical values outlined in the Helsinki Declarations.

S. aureus was isolated on mannitol salt agar (MSA) (Oxoid England) and incubated at 37°C for 24 h. Microbial characterization of the bacterial isolates was based on microbiological methods described by Dionigi et al. (2002). Each distinctive morph type of mannitol-fermenting colony was selected from the MSA plate and sub-cultured on blood agar (Zayo Sigma, Germany) at 37°C for 24 h. Incubated cultures on blood agar were screened using method described by Cowan and Steel (2004). *S. aureus* was identified by colony morphology, Gram stain, DNase catalase and coagulase tests as well as fermentation of mannitol.

Antimicrobial susceptibility testing

The susceptibility of isolates to commonly used antibiotic was determined by the disk diffusion method for *in-vitro* antibiotic susceptibility as described by NCCL (2002), against the following antibiotics; cotrimoxazol (25 µg), cloxacillin (5 µg), erythromycin (5 µg), gentamicin (10 µg), augmentin (30 µg), streptomycin (10 µg), tetracycline (10 µg) and (25 µg), chloramphenicol (10 µg), ofloxacin (5 µg), nalidixic acid (30 µg), nitrofurantoin (20 ug), amoxicillin (25 µg), cotrimoxazole (25 µg) vancomycin (5 µg) and methicillin (5 µg) (Abtek biological Ltd, Liverpool, UK). The concentrations of antimicrobial sensitivity and interpretation of sizes of zones of inhibition were in accordance to Performance Standards for antimicrobial disk susceptibility tests, CLSI (2004) and WHO (2001) breakpoints. The disk diffusion sensitivity was used to determine resistance for both methicillin and vancomycin. The MIC or E-test which are the gold standard for determining vancomycin susceptibility was not used. The method of Hill et al. (2005) was used in identifying multidrug resistant *S. aureus* isolates, the multi antibiotic resistance index (MAR) as well as their plasmid profile were analyzed.

Determination of multi antibiotic resistance (MAR) index

MAR index was calculated for bacteria isolates that showed resistance to more than three antibiotics using the method described by Christopher et al. (2013) and Subramani and Vignesh (2012). This was considered as the number of antibiotics to which tested isolates were resistant to, divided by the total number of antibiotics to which the organism was tested against for sensitivity.

Isolation of plasmid DNA

Plasmid DNA was isolated using the methods described by Birnboim and Doly (1987) with the modifications described by Ombui et al. (2000). All samples were analyzed at the University of Nigeria, Microbiology division. All tubes gel electrophoresis was carried out in tris acetate ETDA buffer containing ethidium bromide for 4 h. A UV transilliminator was used to view the plasmids and photographs taken. Plasmid sizes were estimated from a standard curve drawn of the molecular sizes of the 1.0 Kb distance (Ombui et al., 2000). The strains were grouped based on their molecular weight (MW). Those that had the same MW profile were placed into the same plasmid profile group.

Plasmid curing

Acridine orange treatment method described by Esinome et al. (2010) was used for curing the resistant plasmids of the bacterial isolates.

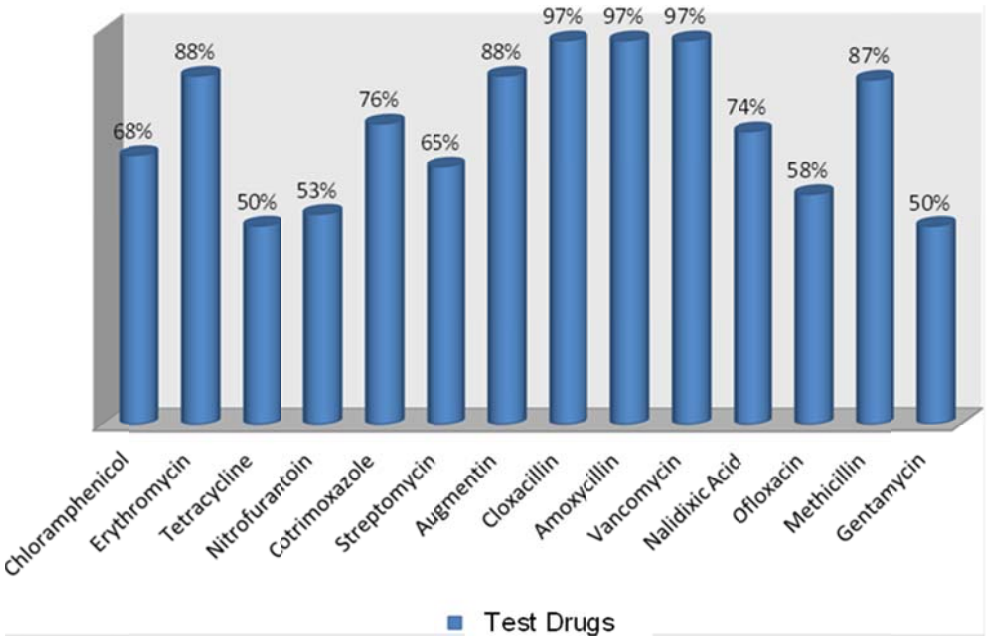

Figure 1. Percentage (%) resistance pattern of isolates to commonly used antibiotics.

Table 1. Multi-antibiotic resistance (MAR) index analysis for 19 *S. aureus* isolates.

Isolates serial number	Number of antibiotic to which isolate was resistant to (a)	MAR Index (a/b)
1	14	1.00
2	13	0.93
3	13	0.93
4	14	1.00
5	14	1.00
6	13	0.93
7	12	0.85
8	14	1.00
9	14	1.00
10	14	1.00
11	13	0.93
12	13	0.93
13	13	0.93
14	13	0.93
15	13	0.93
16	14	1.00
17	14	1.00
18	13	0.93
19	13	0.93

Total number of antibiotics used 14 (b).

RESULTS

A total of 34 specimens were collected and *S. aureus* was isolated from all the samples. Of these, 19 (56%) of the isolates exhibited multidrug resistance to 14 commonly used antibiotics. Eight (42%) of the 19 MDR isolated *S. aureus* were resistant to all the antibiotics while 11(57.89%), exhibited multidrug resistance, sensitive to a maximum of three of the test drugs and the results are as presented in Figure 1. The figure shows resistance to β-lactams [augmentin (88%), cloxacillin (97%) and amoxycillin (97%), aminoglycocides (50 and 65% for gentamycin and streptomycin respectively] and resistance to quinolones [nalidixic acid (74%) and ofloxacin (59%). Resistance was also observed to be high for other antibiotics such as methicillin (87%) and vancomycin (97%) at 5 µg by disc diffusion method. However, this method for the determination of vancomycin resistance is not the gold standard as we compared only concentration effects. The results on the multi-antibiotic resistance (MAR) index analysis for the MDR isolates are as presented in Table 1. All the 19 MDR isolates had a very high MAR index as the calculated values were greater than 0.2. Also, of the 19 MDR isolates screened for plasmids, 18 (94.74%) had plasmids and the profiles are shown in Figures 2 and 3. The plasmid profile sizes ranged between 4936 to 12,130 bp. The isolates where categorized into nine different plasmid profile groups based on their molecular weight (MW) as is shown in Table 2. The most commonly encountered size ranges were 5552 and 6557 bp representing 4 (21%) of the isolates each, whereas 8456 bp were found in three isolates representing 15.8%. When isolates were subjected to curing, 8(44%) lost their plasmids and where cured and the results are as presented in Table 3. The table also shows that there

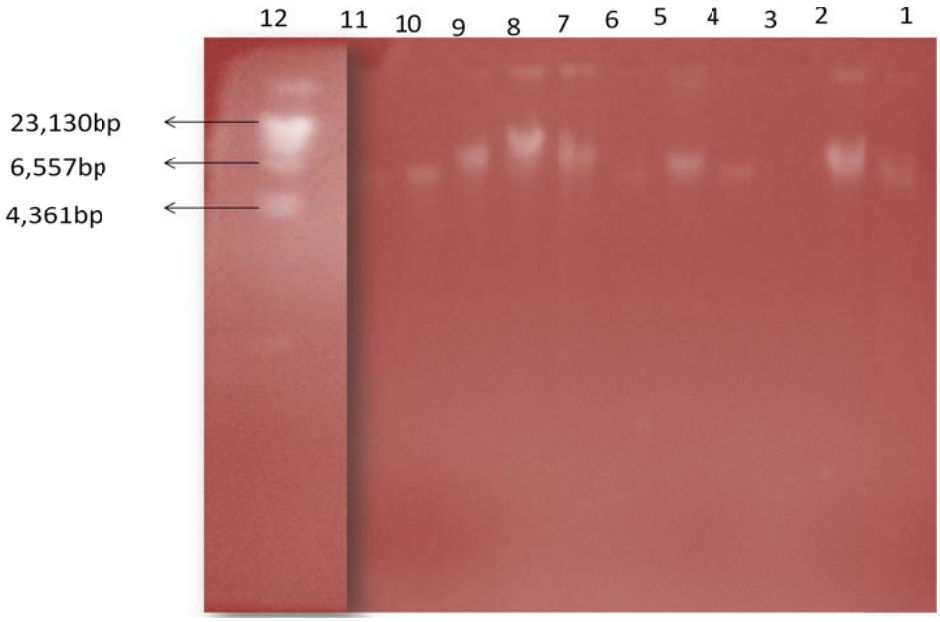

Figure 2. Plasmid profile of *S. aureus* isolates from diabetic wounds showing 10 isolates with plasmids. Lane 12 contains the standard reference molecular weight.

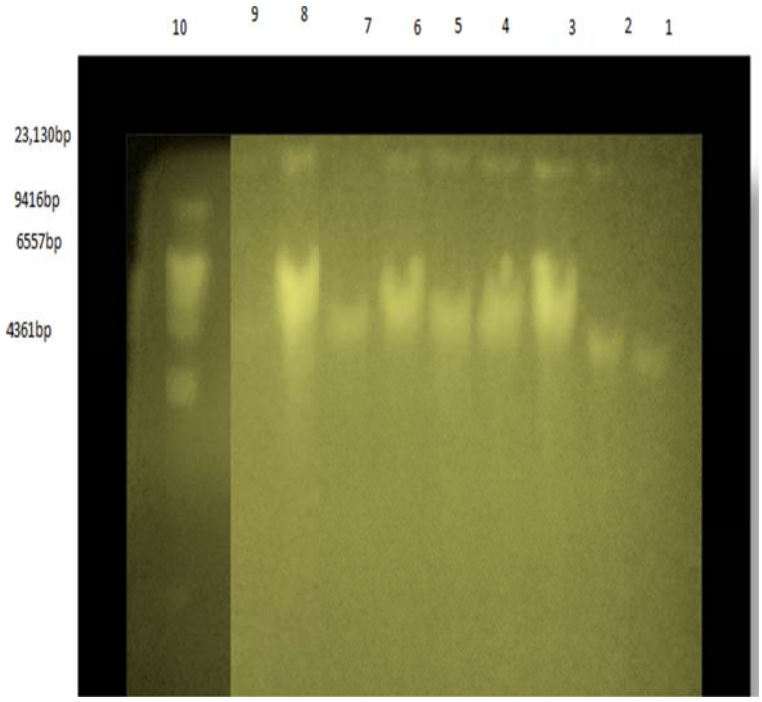

Figure 3. Plasmid profile of *S. aureus* isolates from diabetic wounds showing isolate no. 9 without any plasmid. Lane 10 contains the standard reference molecular weight.

was no correlation between the MW of plasmids and the isolates. Some isolates with the same plasmid MW were cured while others in the same group remained uncured.

DISCUSSION

It is an established fact that bacterial strains, whether

Table 2. Plasmid profile of multidrug resistant *S. aureus* isolates grouped into nine different bands.

Plasmid profile	No. of isolates / (%)	Molecular mass (bp) of isolate
0	1 (5.3%)	None
1	1 (5.3%)	4936
2	4 (21.1%)	5552
3	1 (5.3%)	5861
4	2 (10.5%)	6148
5	1 (5.3%)	6253
6	4 (21.1%)	6557
7	3 (15.8%)	8456
8	1 (5.3%)	9416
9	1 (5.3%)	12,130

Table 3. *S. aureus* isolates with their plasmid profiles against the result of curing with acridine orange.

Sample number	Number of antibiotic resistant (before curing)	MW of plasmids (bp)	Exposure to acridine orange
SA.1	14	5552	+ Growth (not cured)
SA.2	14	6148	+ Growth (not cured)
SA.3	14	5552	+ Growth (not cured)
SA.4	13	6148	+ Growth (not cured)
SA.5	11	5552	+ Growth (not cured)
SA.6	14	6557	+ Growth (not cured)
SA.7	14	6557	+ Growth (not cured)
SA.8	13	12130	-ve Growth (cured)
SA.9	13	5552	-ve Growth (cured)
SA.10	14	4936	-ve Growth (cured)
SA.11	13	5861	-ve Growth (Cured)
SA.12	13	6253	+ Growth (not cured)
SA.13	12	8456	+ Growth (not cured)
SA.14	14	8456	-ve Growth (cured)
SA.15	14	6557	-ve Growth (cured)
SA.16	10	8456	+ Growth (not cured)
SA.17	13	6557	-ve Growth (cured)
SA.18	12	9416	-ve Growth (cured)
SA.19	14	No plasmid	+ Growth (not cured)

Gram-positive or -negative exhibit resistance to antibiotics worldwide thus creating an unprecedented public health threat. This resistance can sometimes be plasmid base. With the problems of healthcare funding in the developing world, most health care centers still prescribe antibiotics without necessary clinical investigations.

Misuse of antibiotics was reported in South-eastern Nigeria were the present investigation was carried out (Esimone et al., 2007). This behaviour could lead to the spread of MDR bacteria and consequently a change in antibacterial resistance pattern. The finding of plasmids observed in 18 of the 19 MDR isolates in the present study suggests a plasmid based resistance. It would not be unexpected that these patients presenting with wounds at the hospital would probably have tried self medication and have only gone to the health center probably as a last resort. Also, the 19 MDR *S. aureus* diabetic isolates met the criterion of being described as MAR according to the definition of Hill et al. (2005), as they exhibited resistance to the following three classes of antibiotics; β-lactam, aminoglycosides and quinolones. The high level of resistance observed to β-lactam antibiotics in the present study is similar to the findings of

other workers (Chen et al., 2006; Adegoke and Komolafe, 2009). This characteristic high level of *S. aureus* resistance to β-lactam antibiotics is said to be plasmid based (Adegoke and komolafe, 2009). Earlier report stipulated that R-plasmid-mediated antibiotic can spread in an area where there is heavy use of antibiotics (Subramani and Vignesh, 2012; Daini et al., 2006). Nigeria lies in the region of high antibiotic misuse as reported by Esinome et al. (2007).

The exhibition of MDR to most commonly used antibiotics by the isolates in this study could suggest the possibility of MRSA presence in this region. However there is the need for further investigation to confirm this danger.

According to a recent report, MRSA was isolated from human specimens in another area of South-eastern region of Nigeria (Chibuike et al., 2014). It is therefore important to keep a watch on the emergence of MDR superbugs in this region of the world. Plasmid sizes ranged between 4936 to 12130 bp. This finding is not consistent with the work of Esimone et al. (2010) who reported plasmid size range from 11000-18000 bp. Also Daini and Akano (2006) reported plasmid szes of 1.26, 23.13 and 25.12 kb, while plasmid size range of 300-4000 bp was recorded by Chibuike et al. (2014). This therefore indicates that there is variability in plasmid sizes of MDR S. *aureus*.

Also, MW sizes as seen in the present study were not consistent with curing among the 18 isolates with plasmids as each isolates exhibited individual characteristics. Variations in the methods of isolation could have contributed to these differences. Barton et al. (1995) commented on the difficulty in establishing the size limits of plasmids or the real size distribution in any organism because of methods used in isolation of the organisms as well as the characteristics displayed.

The present findings of MDR *S. aureus* while not appearing to be a usual phenomenon in Nigeria, is a worrisome situation. The 94.7% of the MDR isolates in the present study containing plasmids is high when compared with the 41.2% reported by other workers (Diani and Akano, 2009). This further highlights the pressing need to keep a watch on MDR resistant bacteria emerging from this region. Also the result on plasmid curing as seen in the present study means that antibiotic resistance cannot be said to be entirely plasmid mediated.

These findings are similar to those of Chibuike et al. (2014) and contrary to the findings of Daini and Akano (2009) who also reported a complete cure amongst their isolates. No explanation can be given for now, as to why some plasmids with same MW group were cured while others remained uncured.

There is the possibility that resistance for many were plasmid base while the absence of plasmid in one could mean that the some might be chromosomal based. The possibility of the patients self-medicating cannot be over-looked, as well as the over-zealousness to treat every infection with antibiotics by hospital doctors or by the patients.

Antibiotics can easily be bought off the shelves in area of study. Therefore, the high levels of MDR *S. aureus* isolates observed in the present study could be as a result of misuse or undue exposure to antibiotics. Also, for all the isolates in the present investigation, bacterial MAR index was greater than 0.2, implying that the strain of bacteria originated from an environment of high use antibiotics as indicated by Christopher et al. (2013) and Subramani and Vignesh (2012).

Conclusion

The high level of multi drug resistance *S. aureus* observed in this study could potentially predict a change in resistance pattern of the community. It also suggests that the patients involved in this study are likely from a region of high antibiotic use. This study therefore highlights once again that misuse of antibiotics could possibly lead to a change in microbial resistance characteristics causing treatment failure and increase in the cost of infection control. The fact that the populace from this region can easily buy antibiotics without prescription lays credence to this and therefore such behaviour could lead to a major public health issue that needs to be attended to urgently.

Conflict of Interest

The author(s) have not declared any conflict of interest.

REFERENCES

Adegoke AA, Komolafe AO (2009). Multi-drug resistant *Staphylococcus aureus* in clinical cases in Ile-Ife, Southwest Nigeria. Int. J. Med. Med. Sci. 1(3):068-072.

Adeleke OE, Odelola HA, Oluwole FA (2002). Curing of Antibiotic resistance in clinical strains of *Staphylococcus aureus*. Afr. J. Med. Pharm. Sci. 6:19-25.

Akinyemi KO, Alabi SA, Taiwo MA, Omonigbehin EA (1997). Antimicrobial susceptibility pattern and plasmid profiles of pathogenic bacteria isolated from subjects with urinary tract infections in Lagos Nigeria. Nig. Qt. J. Hosp. Med. 7:7-11.

Barton BM, Harding GP, Zuccarelli AJ (1995). A general method for detecting and Sizing Large Plasmids. Anal. Biochem. 226(2): 235-40.

Bhakta M, Arora S, Bal M (2003). Intra-species Transfer of a Chloramphenicol- resistance plasmid of staphylococcal origin. Indian J. Med. Res. 117:146-151.

Birnboim HC, Doly J (1987). A. rapid alkaline extraction procedure Salmonellosis traced to pasteurised milk. J. Amer. Med. Assoc. 258:3269-3274.

Brennan GI, Shore AC, Corcoran S, Tecklenborg S, Coleman DC, O'Connell B (2012). Emergence of hospital- and community-associated panton-valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* genotype ST772-MRSA-V in Ireland and detailed investigation of an ST772-MRSA-V cluster in a neonatal intensive care unit. J. Clin. Microbiol. 50(3):841-847.

Chen KT, Huard RC, Della-Latta P, Lisa S (2006). Prevalence of methicillin-sensitive and methicillin-resistant Staphylococcus pregnant women. Obstet. Gynecol. 108:482-287.

Chibuike I, Reginald AO, Solomon CU (2014). Antibiotic resistant pattern

and plasmid profiles of methicillin resistant *Staphylococcus aureus* isolated from human samples. Br. Microb. Res. J. 4(2):185-194.
Christopher AF, Hora S, Ali Z (2013). Investigation of plasmid profile, antibiotic susceptibility pattern multiple antibiotic resistance index calculation of *Escherichia coli* isolates obtained from different human clinical specimens at tertiary care hospital in Bareilly - India. Ann. Trop. Med. Public Health 6:285-289.
Clinical Laboratory Standards Institute (CLSI) (2004). Performance standards for antimicrobial susceptibility testing; fourteenth informational supplement (M100-S14). 24.
Cowan ST, Steel KJ (2004). In Manual for identification of Medical Bacteria. 3rd Edition.Cambridge University Press. pp. 50-140.
Daini AO, Akano SA (2009). Plasmid Mediated antibiotic resistance in *Staphylococcus aureus* from patients and non patients. Sci. Res. Essay 4(4):346-350.
Daini OA, Ogbolu DO, Ogunledun A (2006). Plasmid determined resistance to Quinolones in Clinical Isolates of Gram-negative Enteric Bacilli. Afr. Med. Sci. 35: 437-441.
De Vriese AS, Vandecasteele SJ (2014). Vancomycin: the tale of the vanquisher and the pyrrhic victory. Perit. Dial. Int. 34(2):154-61.
Dhanoa A, Singh VA, Mansor A, Yusof MY, Lim KT, Thong KL (2012). Acute haematogenous community-acquired methicillin-resistant *Staphylococcus aureus* osteomyelitis in an adult: Case report and review of literature. BMC Infect. Dis. 12:270.
Diep BA, Chambers HF, Graber CJ et al. (2008). Emergence of multi-drug - resistant community - associates Methicillin - resistant *Staphylococcus aureus.* Clone USA 300 in Men who have sex with men. Ann. Intern. Med. 148:1-17.
Dionigi R, Rover F, Dionigi G, Imperatori A et al. (2002). Risk factors in surgery. J. Chemother. 13:6-11.
Esimone CO, Nworu CS, Udeogaranya PO (2007). Utilization of antimicrobial agents with and without prescriptions by outpatients in selected pharmacies in south-eastern Nigeria. Pharm. World Sci. 29(6):655-660.
Esinome CO, Nwosu CS, Harrison GT (2010). Antibiogram and plasmid profile of some Multi - Antibiotics resistant Urinopathogens obtained from local communities of Southeastern Nigeria. Ibnosina J. Med. Biomed. Sci. 2(4):152-159.
Gould IM, David MZ, Esposito S, Garau J, Lina G, Mazzei T, Peters G (2012). New insights into methicillin-resistant *Staphylococcus aureus* (MRSA) pathogenesis, treatment and resistance. Int. J. Antimicrob. Agents 39(2):96-104.
Hill D, Barbara R, Aniko P, et al. (2005). Antibiotic Susceptibilities of *Pseudomonas aeruginosa* Isolates Derived from Patients with Cystic Fibrosis under aerobic anaerobic and biofilm Conditions. J. Clin. Microbiol. 43:5085-5090.
King MD, Humphrey BJ, Wang YF et al. (2006). Emergence of community - acquired methicillin -resistant *Staphylococcus aureus* USA 300 clone as the predominant cause of skin and soft tissue infections. Ann. Intern. Med. 144: 309-317.
Mammina C, Calà C, Bonura C, et al. (2012). EPI-MRSA Working Group: Polyclonal non multi-resistant *Staphylococcus aureus* isolates from clinical cases of infection occurring in Palermo, Italy, during a one-year surveillance period. Ann. Clin. Microbiol. Antimicrob.11: 17.
Mishra SK, Rijal BP, Pokhrel BM (2013). Emerging threat of multidrug resistant bugs -*Acinetobacter calcoaceticus baumannii* complex and Methicillin resistant *Staphylococcus aureus.* BMC Res. Notes 6(98):2-6.
NCCL (2002). National Committee for Clinical Laboratory Standards for antimicrobial disk susceptibility testing. Twelfth information supplement (M100 - S12) Wayne, PA.
Ombui JN, Kimotho AM, Nduhiu JG (2000). Antimicrobial Resistant patterns and Plasmid profiles of *Staphylococcus aureus* isolated from milk and meat. E. Afr. Med. J. 77(9): 463-465
Park B, Liu GY (2012). Targeting the host-pathogen interface for treatment of *Staphylococcus aureus* infection. Semin. Immunopathol. 34(2):299-315.
Subedi S, Brahmadathan KN (2005). Antimicrobial susceptibility patterns of clinical isolates of Staphylococcus aureus in Nepal. Clin. Microbiol. Infect. 11(3): 237-240.
Subramani S, Vignesh A (2012). MAR index study and MDR Character analysis of a few golden Staphylococci isolates. Asian J. Pharm. Life Sci. 2(2): 151-154.
World Health Organization (2001). Improve and expand surveillance. Antibiotic resistance: synthesis of recommendations by expert policy groups. Switzerland: Geneva; 2001. pp. 15-31.