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Full Length Research Article

OCCURRENCE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM PATIENTS ATTENDING SOME HOSPITALS WITHIN KADUNA METROPOLIS

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ABSTRACT
The global spread of methicillin resistant *Staphylococcus aureus* (MRSA) constitute one of the most serious contemporary challenges to the treatment of hospital-acquired infections. This study was carried out to determine the occurrence of *clFA* gene and antibiotics susceptibility pattern of *Staphylococcus aureus* from clinical samples of patients attending selected hospitals within Kaduna metropolis. One hundred and eighty (180) clinical samples were collected from four selected hospitals and *S. aureus* was isolated and identified using standard methods. The study showed that 25(41.7%) of *Staphylococcus aureus* (*S. aureus)* were isolated from wound specimens, 15(25%) from urine samples and high vaginal swabs (HVS) has the least percentage of 12(20%). Isolates were susceptible to Vancomycin, Ciprofloxacin and Erythromycin but shows resistant to Methicillin, Gentamycin and Ampiclox. Vancomycin was the most effective antimicrobial agent against *S. aureus*. The presence of *clFA* gene was detected via Polymerase Chain Reaction (PCR) and agarose gel electrophoresis. MRSA was isolated with higher percentage in wound swab 8(32%), urine sample 4(26%) and HVS has the least of 3(25%). This study recommend regular surveillance of hospital associated infections and monitoring antibiotic susceptibility pattern and strict drug policy for antibiotics used within and outside the hospital environment.

Keywords: Methicillin, Resistant, *Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus is facultative anaerobic, Gram positive cocci which looks as grape-like clusters when observed through the microscope they produce big, round, golden-yellow colonies, when grown in mannitol salt agar. The golden presence is the etymological root of the bacteria name, *aureus* meaning "golden" in Latin (Mani *et al*., 2016).

Staphylococcus aureus grows in big, round, opaque colonies at an optimum temperature of 37^{o}C, though it can grow anywhere between 10 to 46^{o}C (Velasco *et al.,* 2017). The growth is supplemented in the presence of oxygen and carbon (IV) oxide. They digest proteins and lipids and they are accomplished of fermenting sugar (Berke and Tilton, 2016).

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S. aureus causes wide range of infections in human. The clinical infections of *S. aureus* are classified into community and nosocomial categories based on origin of infection (Silver *et al.,* 2015). These two types are distinct in clinical manifestations of the infections, antibiotic susceptibility and the genetic background of the infecting *S. aureus* strains (Rao *et al*., 2017). For decades, *S. aureus* has been predominately a nosocomial pathogen and is a leading cause of mortality and morbidity in hospitals (Rao *et al.,* 2017). The important clinical *S. aureus* infections are bacteraemia, infective endocarditis, skin and soft tissue infections. Other clinical infections are epidural abscess, meningitis, toxic shock syndrome and urinary tract infections (Robertson *et al.,* 2010).

The emergence and spread of methicillin-resistant *S. aureus* (MRSA) strains which are often multi-drug resistant in hospitals and subsequently in community resulted in significant mortality and morbidity. The epidemiology of MRSA has been evolving since its initial outbreak which necessitates a comprehensive medical approach to tackle this pathogen. Vancomycin has been the drug of choice for years but its utility was challenged by the emergence of resistance. In the last 10 years or so, newer anti-MRSA antibiotics were approved for clinical use. However, being notorious for developing antibiotic resistance, there is a continuous need for exploring novel anti-MRSA agents from various sources including plants and evaluation of non-antibiotic approaches (Silver *et al.,* 2015).

Staphylococcus aureus is a major cause of nosocomial and community-acquired infection. It expresses several factors that promote avoidance of phagocytosis by polymorphonuclear leucocytes (Patti *et al.,* 2012). Clumping factor A (*clFA*) is a fibrinogen-binding surface protein of *S. aureus* that is an important virulence factor in several infection models (Francois *et al.,* 2014).

MATERIALS AND METHODS

Study Area

This study was carried out at Kaduna metropolis, Northwest Nigeria. Kaduna State consists of 23 local Government Areas and 59 ethnic groups with a population of 1652800. Their major occupation is trading, farming and rearing of cattle. Kaduna State is located on latitude 10.53^{o}N and longitude 7.44^{o}E with an elevation of 626m above sea level. It has an annual rainfall range of between 200mm to 600mm (Abaji *et al.,* 2010).

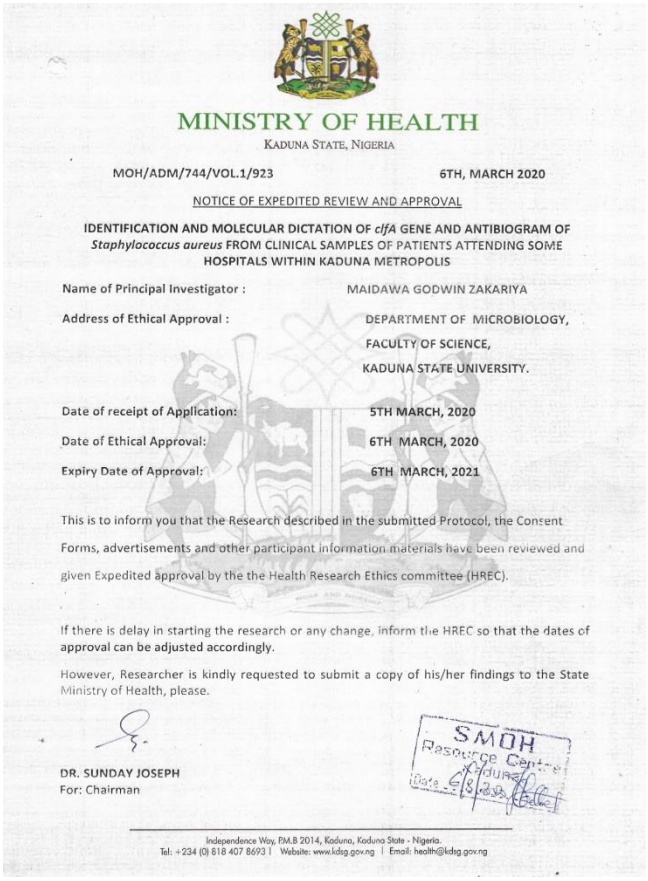
Study Site

Four hospitals were selected for the study; Barau Dikko Teaching Hospital (Located at Lafia Road, City Centre in Kaduna North Local Government Area), Yusuf Dantsoho Memorial Hospital (Located at Tudun Wada in Kaduna South Local Government Area), Gwamna Awan General Hospital, Kakuri (Located at Block 4, Kakuri in Kaduna South Local Government Area) and General Hospital Sabo Tasha (Located at Sabon Tasha ward, Opposite NNPC Quarters Unguwan Boro in Chikun Local Government Area).

Study Population
Males and females patients attending Barau Dikko Teaching Hospital, Yusuf Dantsoho Memorial Hospital, Tudun Wada, Gwamna Awan General Hospital, Kakuri and General Hospital Sabo Tasha were used as the study population.

Study Design
A prospective study was carried at four selected hospitals at Kaduna metropolis through voluntary and confidential consent from the patients' parents.

Ethical Approval
Ethical approval was obtained by Ethical Committee of the Kaduna State Ministry of Health (MOH/ADM/744/VOL.1/923) and Barau Dikko Teaching Hospital Kaduna before the commencement of the study.



MINISTRY OF HEALTH
KADUNA STATE, NIGERIA

MOH/ADM/744/VOL.1/923 6TH, MARCH 2020

NOTICE OF EXPEDITED REVIEW AND APPROVAL

IDENTIFICATION AND MOLECULAR DICTATION OF *clfA* GENE AND ANTIBIOGRAM OF *Staphylococcus aureus* FROM CLINICAL SAMPLES OF PATIENTS ATTENDING SOME HOSPITALS WITHIN KADUNA METROPOLIS

Name of Principal Investigator : MAIDAWA GODWIN ZAKARIYA

Address of Ethical Approval : DEPARTMENT OF MICROBIOLOGY, FACULTY OF SCIENCE, KADUNA STATE UNIVERSITY.

Date of receipt of Application: 5TH MARCH, 2020

Date of Ethical Approval: 6TH MARCH, 2020

Expiry Date of Approval: 6TH MARCH, 2021

This is to inform you that the Research described in the submitted Protocol, the Consent Forms, advertisements and other participant information materials have been reviewed and given Expedited approval by the the Health Research Ethics committee (HREC).

If there is delay in starting the research or any change, inform the HREC so that the dates of approval can be adjusted accordingly.

However, Researcher is kindly requested to submit a copy of his/her findings to the State Ministry of Health, please.

DR. SUNDAY JOSEPH
For: Chairman

SMOH Resource Centre Kaduna

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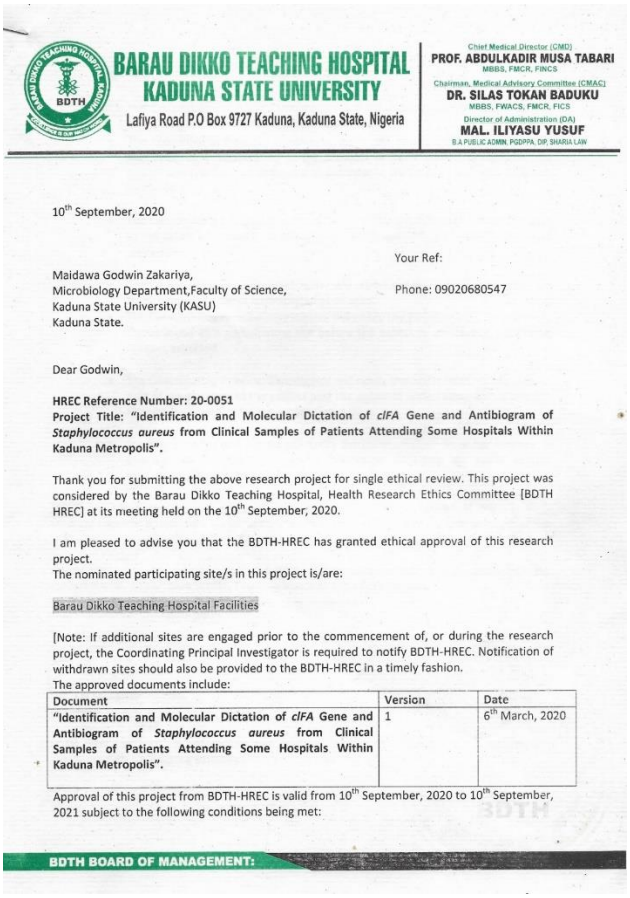
Tel: +234 (0) 818 407 8693 | Website: www.kdsg.gov.ng | Email: health@kdsg.gov.ng

Figure 1. Ethical Approval from Kaduna State Ministry of Health



BARAU DIKKO TEACHING HOSPITAL
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Chief Medical Director (CMD)
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10th September, 2020

Your Ref:

Maidawa Godwin Zakariya,
Microbiology Department,Faculty of Science,
Kaduna State University (KASU)
Kaduna State.

Phone: 09020680547

Dear Godwin,

HREC Reference Number: 20-0051
Project Title: "Identification and Molecular Dictation of *clfA* Gene and Antibiogram of *Staphylococcus aureus* from Clinical Samples of Patients Attending Some Hospitals Within Kaduna Metropolis".

Thank you for submitting the above research project for single ethical review. This project was considered by the Barau Dikko Teaching Hospital, Health Research Ethics Committee [BDTH HREC] at its meeting held on the 10th September, 2020.

I am pleased to advise you that the BDTH-HREC has granted ethical approval of this research project.
The nominated participating site/s in this project is/are:

Barau Dikko Teaching Hospital Facilities

[Note: If additional sites are engaged prior to the commencement of, or during the research project, the Coordinating Principal Investigator is required to notify BDTH-HREC. Notification of withdrawn sites should also be provided to the BDTH-HREC in a timely fashion.
The approved documents include:

Document	Version	Date
"Identification and Molecular Dictation of *clfA* Gene and Antibiogram of *Staphylococcus aureus* from Clinical Samples of Patients Attending Some Hospitals Within Kaduna Metropolis".	1	6th March, 2020

Approval of this project from BDTH-HREC is valid from 10th September, 2020 to 10th September, 2021 subject to the following conditions being met:

BDTH BOARD OF MANAGEMENT:

Figure 2. Ethical Approval from Barau Dikko Teaching Hospital Kaduna State University

Sample Collection
A total of one hundred and eighty (180) clinical samples (60 samples each from wound swab, urine and high vaginal swab) were obtained from the patients with suspected *Staphylococcus aureus* infections attending the four selected hospitals of Kaduna metropolis, Nigeria. The samples were transported to Laboratory of Microbiology Department, Kaduna State University, Kaduna, Nigeria.

Media Preparation
Mannitol Salt Agar (MSA) was measured according to manufacturer's directive using and analytical balance. Sterile distilled water was added to the measured medium in a conical flask. It was boiled to dissolve the medium in the flask by using hot plate. Cotton and foil paper was used to cover the mouth of the flask. The medium was sterilized using autoclave at 121°C for 15minutes. The medium was removed from the autoclave and allow to cool on the table. It was dispense into the petri plate and allow to solidify.

Isolation of *Staphylococcus aureus*
Ten (10ml) of urine samples were centrifuged at 2000rpm for 10mins in a centrifuge and the supernatant was discarded. 0.5ml from the deposit was transferred using pasture pipette onto the prepared MSA. Sterilized glass rod spreader was used to spread the samples on the surface of the media and allowed to absorb for

30 min. The wound swabs and the high vaginal swabs (HVS) were spread on the surface of the media and allowed to absorb for 30 min. The plates were incubated at 37^{o}C for 24h and were examined for bacterial growth. This was done in duplicate as described by (Ghenghesh, 2016).

Biochemical Test

All the recovering isolates that showed golden yellow colonies on mannitol salt agar plates were confirmed by a biochemical test using standard bacteriological procedure according Cheesbrough (2010), which includes catalase and coagulase test.

Antibiotic Susceptibility Test

Antimicrobial susceptibility test was carried out on the confirmed *Staphylococcus aureus* using a modified Kirby-Bauer method. Briefly, a bacterial suspension adjusted to 0.5 MacFarland was inoculated onto chocolate agar (Oxoid, UK). Commercially prepared antibiotics (Oxoid, UK) disc containing 7 different antibiotics namely; Gentamycin (10µg), Vancomycin (15µg), Erythromycin (15µg), Ampiclox (10µg), Amoxicillin (30µg), Methicillin (10µg) and Ciprofloxacin (5µg). All the plates were incubated at 37^{0}C for 24h. To measure zones of inhibition the plates were held a few inches above a black, non-reflective surface illuminated with reflected light, a ruler was used with the unaided eye while when viewing the back of the Petri dish. (CLSI, 2014). The result was recorded and was compared with the zone of inhibition diameter interpretive standard of the Clinical Laboratory Standard Institute (CLSI, 2014).

Screening of MRSA among the *Staphylococcus aureus*

Fifty two (52) positives samples for *S. aureus* were further subjected to antibiotic testing to determine the occurrence of methicillin resistant *Staphylococcus aureus* using cefoxitin disk diffusion method (Coombs *et al.,* 2013).

Confirmation of Methicillin S*taphylococcus aureus* using Genus Specific *clFA* gene by PCR Isolation of DNA

The enriched cultured was centrifuged at 12,000 rpm for 5 mins. The pellet were resuspended in sterile distilled water. The mixture was boiled using heat block for 10 min and immediately cooled at -20^{0}C for 10 min. the supernatant was used as a source of template DNA for PCR (Martineau *et al*., 2017).

Primers set and PCR amplification conditions

The primer for *clFA* with 300bp by Life-river (2018) was used for the PCR having the following sequence 5'-F-GTGGCTTCAGTGCTTGTAGGTA-3' and 3'-TGCTTGATTGAGTTGTTGCCG- 5'. Single cell PCR was first optimized. PCR for amplification of *clFA* gene was then perform using 12.5 µl of Tag green Master Mix 2x DNA polymerase, specific primers foe *clFA* (1 µl forward and 1 µl reverse) , 2 µl of DNA extract as a template 8.5 µl of nuclease free water to make the final reaction water volume up to 25 µl. the PCR condition was run with the initial denaturation at 95^{0}C for 2 min,34 cycles of denaturation at 95^{0}C for 15 secs, 45^{0}C for 1 min, and 75^{0}C for 45 sec and final extension at 72^{0}C for 7 min (Rahn *et al,* 1992).

Agarose gel electrophoresis

The PCR products were analysed on the 1.2% w/v agarose gel stained with ethidium bromide according to the methods adopted by (Martineau *et al*., 2017). Eight (8) µl of PCR product was mixed with 3 µl of 6x loading dye were loaded on to agarose gel. A 100bp ladder was used as a ruler. Then the current of 120 volts was applied for 30 minutes. The products were visualized under UV light fitted documentation system.

RESULTS

Frequency of occurrence of *Staphylococcus aureus* from the clinical samples was studied and result showed in Table 1. The result indicated that wound swab had higher frequency of occurrence of *Staphylococcus aureus* 25(41.7%), followed by Urine samples with occurrence of 15(25%) and high vaginal swab had the lowest frequency occurrence of *Staphylococcus aureus* of 12(20.0%).

The morphological and biochemical characterization of *Staphylococcus aureus* isolates from the clinical samples showed that the organism was Gram positive cocci, grape-like cluster when observed through the microscope, the produce big, round, golden-yellow colonies on mannitol salt agar. They were coagulase positive, catalase positive, VP positive, citrate positive, oxidase and indole negative (Table 2).

The susceptibility pattern of gram positive bacteria was tested against seven different antibiotics such as Gentamycin, Vacomycin, Ampiclox, Streptomycin, Methicillin, Erythromycin and Ciprofloxacin. The study showed high resistant to Ampiclox, Gentamycin and Erythromycin while high sensitive was showed in Vancomycin as showed in Table 3.

Table 4 shows the frequency occurrence of Methicillin resistance *Staphylococcus aureus* (MRSA) among the *Staphylococcus aureus* isolated from the different clinical samples. The result indicated that wound swab had higher frequency of occurrence of *Staphylococcus aureus* 8(32.0%), followed by Urine samples with occurrence of 4(26.7%) and high vaginal swab had the lowest frequency occurrence of *Staphylococcus aureus* of 3(25.0%).

The isolated *Staphylococcus aureus* was confirmed by PCR using reported specific *clFA* gene which is a fibrinogen-binding surface protein of *S. aureus* that is an important virulence factor in several infection models (Francois *et al.,* 2014). There was PCR amplification with the primer used at the expected 300 bp confirming *Staphylococcus aureus* (Figure. 1).

Table 1: Frequency Occurrence of Bacteria Isolate from Different Hospitals in Kaduna Metropolis

Hospitals	No. of Samples Analyzed	No. of Positive Samples	Frequency Occurrence (%)
B1	45	11	24
Y2	45	9	20
G3	45	17	37
S4	45	15	33
Total	180	52	

Keys - B1 Barau Dikko Teaching Hospital
Y2 Yusuf Dantsoho Memorial Hospital
G3 Gwamna Awang Hospital
S4 General Hospital Sabo

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Table 2: Morphological and Biochemical Characteristics of Suspected Bacteria Isolate obtained from Clinical Sample in selected Hospitals within Kaduna Metropolis

Characteristics	*Staphylococcus aureus*
Color	Golden yellow on mannitol salt agar
Shape	Cocci
Gram reaction	+
Biochemical test	
Coagulase	+
Catalase	+
Citrate	+
Indole	-
Voges-Proskauer	+
Oxidase	-

Keys – Negative
+ Positive

Table 3: Percentage Antibiotics Susceptibility Pattern of *Staphylococcus aureus* Isolated from Clinical Samples (N=52)

Serial No.	Antibiotic/Disc Potency	Resistant {N (%)}	Intermediate {N (%)}	Sensitive {N (%)}
1	Ciprofloxacin(10µg)	10(19.2)	9(17.3)	33(63.4)
2	Gentamycin(30µg)	15(28.8)	5(9.6)	32(61.5)
3	Ampiclox(30µg)	16(30.7)	8(15.3)	28(53.8)
4	Methicillin(10µg)	14(26.9)	4(7.6)	34(65.3)
5	Erythromycin(10µg)	5(9.6)	12(23.0)	35(67.3)
6	Vancomycin (10µg)	2(3.8)	8(15.3)	42(80.7)
7	Streptomycin(30µg)	8(15.3)	10(19.2)	34(65.3)

Table 4: Occurrence of Methicillin Resistant *Staphylococcus aureus* from Different Clinical Samples

Clinical sample	Number of Isolates	Number of MRSA (%)
Wound swab	25	8 (32.0)
Urine	15	4 (26.7)
HVS	12	3 (25.0)
Total	52	14 (26.9)

Key: HVS=High Vaginal Swab

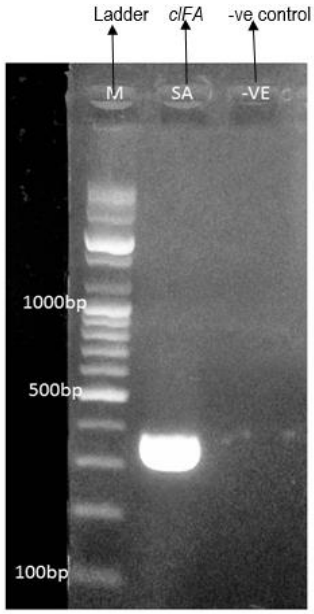

Plate 4.1: Agarose Gel Electrophoresis of Amplified *clFA* gene of *Staphylococcus aureus* (300bp)

DISCUSSION

The appearance of yellow color of *S. aureus* on Mannitol Salt Agar (MSA) is as a result of the organism been able to utilize mannitol leading to production of acid which result in changing the color of the medium from pink to yellow, this has been reported by Robinson and Enright (2014) to be as a result of fermentation of mannitol salt and consequent production of acid. The organism is Gram positive cocci appearing in grape-like Peng *et al.* (2015) has reported similar appearance of *S. aureus,* the biochemical characteristics of this organism where catalase and coagulase positive and produced β-hemolysis on blood agar plate. In this study, the majority of *S. aureus* strains of (32%) were isolated from wound specimens. The second higher percentages were isolated from urine specimens (26%), while the bacterial strains in the high vaginal swabs were lower (Table 4.3). These results are compatible with different previous results reported in other parts of world (Manikandan *et al.,* 2016; Mani *et al.,* 2016; Rao *et al.,* 2017). Similar finding also shown higher frequency *S. aureus* in the wound specimen more than any other clinical specimen as reported by (Orret *et al.,* 2016).

Vancomycin was the antibiotic that has effect against most isolates in this study (80.8%), which makes it a drug of choice for treating multi-drug resistant MRSA. Streptomycin was the second effective antibiotic after Vancomycin (69.2%), similar to the report from Iraq (Babakir *et al.,* 2012). It followed by Erythromycin and streptomycin (80 % to 88.9 %, respectively). Lower sensitivity to rifampicin has been reported by other authors (43% and 43.7%), and chloramphenicol (6.1 %) (Babakir *et al.,* 2012). Hafeez and Aslam (2016) reported higher percentage of MRSA (40 %) in high vaginal swabs. However, in this study MRSA were not detected in the same specimens, but MRSA were isolated in higher percentage in wound swabs. Coombs *et al*., (2013) reported that MRSA have been isolated in lower percentage from urine specimens, these variations may be related to many reasons such as the patients' population, types of skin normal flora, specimens collection procedures and number of specimens used.

Conclusion

Out of one hundred and eighty (180) clinical samples that were collected, 52 samples were positive given the percentage occurrence of *S. aureus* as 28.9%. The antibiotic susceptibility pattern of *S. aureus* was determined and vancomycin was the most effective antimicrobial agent in this study.

Samples obtained from wound swabs has the highest percentage occurrence with 41.7% and HVS has the less occurrence with 20%. MRSA was isolated with higher percentage in wound swab 8(32%), urine sample 4(26%) and HVS has the least of 3(25%). Clumping factor A (*clFA*) gene was detected in methicillin resistant *Staphylococcus aureus* isolates via Polymerase Chain Reaction (PCR) and agarose gel electrophoresis.

Recommendations

1. There should be regular surveillance of hospital associated infections including monitoring antibiotic sensitivity pattern.
2. A comprehensive tracking system should capture data on emerging antimicrobial resistant trends, report infections from different healthcare sectors and veterinary care across the country, and recognize high risk patients, among others.
3. *in-vitro* susceptibility testing of every isolate of MRSA in the clinical laboratories may be helpful for reducing the incidence of these infections.
4. Reevaluation of existing infection control practices, implementation of more effective practices (screening of MRSA carriers, isolation of patients, colonized healthcare workers, and environmental decontamination, among others) should be a priority.

REFERENCES

Abaji, I.B., Ishaya S. and Usman S.U. (2010). An analysis of rainfall trends in Kafanchan, Kaduna State Nigeria. *Research Journal of Environment and Earth Sciences,* 2(2): 89-96

Babakir, M.M., Othman, N., Najmuldeen, H.H., Noori, C.K., Fatah, C.F. and Perno, C.F. (2012). Antibiotic susceptibility of vancomyin and nitrofurantoin in *Staphylococcus aureus* isolated from burnt patients in Sulaimaniyah, Iraqi Kurdistan. *The Journal of Hospital Infection,* 35:439-446.

Berke, A. and Tilton, R.C. (2016). Evaluation of rapid coagulase methods for the identification of Staphylococcus aureus. *Journal of Clinical. Microbiology*, 23(5): 916-919.

Cheesbrough, M. (2010). District Laboratory practice in Tropical Countries. 2nd Edition, Cambridge University Press, Cambridge, United Kingdom, 116-245.

Clinical and Laboratory Standard Institute (CLSI) (2014). Performance standard of antimicrobial disk susceptibility tests; Twenty Fourth Informational Supplement; M02- A11, MO7-A9 AND M11-A8. Clinical and Laboratory Standard Institute 950. West Valley Road, Suite 2500 Wayne, PA 19087 United State of America Geveva.

Coombs, G.W., Goering, R.V., Chua, K.Y., Monecke, S. and Howden, B.P. (2013). A comparative analysis of community acquired and hospital acquired methicillin resistant *Staphylococcus aureus. Journal of Clinical and Diagnostic Research,* 7:1339-42.

Dilnessa, T., Bitew, A. and Lin, B. (2016). Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* isolated from clinical samples at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia, *BMC Infectious Diseases*, **16**(1): 398-399

Francois P., Vaudaux P., McDevitt D. and Foster T. J. (2014). Molecular characterization of the clumping factor (fibrinogen receptor) of *Staphylococcus aureus*. *Journal of Molecular Microbiology,* 11:237–248

Ghenghesh, K.S. (2016). Methicillin-resistant *Staphylococcus aureus* (MRSA) in a tertiary surgical and trauma hospital in Benghazi, Libya. *Journal of Infection in Developing Countries*, 5(10): 723–726.

Hafeez, C.A. and Aslam, M. (2016). Prevalence and Antimicrobial Susceptibility of Methicillin Resistant *Staphylococcus aureus* (MRSA). *Journal of Clinical and Diagnostic Research,* 57:245-232

Mani, K.R., Rajaduraipandi K., Mani M. and Manikandan P. (2016). Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus*: a multi-center study. *Indian Journal of Medical Microbiology*, 24:34-38.

Manikandan, P., Mani, K.R., and Bhaskar, M. (2016).Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus*: a multicentre study. *Indian of Medical Microbiology,* 24:34-38.

Martineau, F., Picard F.J., Roy P.H., Ouellette M and Bergeron M.G. (2017). Multiplex PCR assays for the detection of clinically relevant antibiotic resistance genes in staphylococci isolated from patients infected after cardiac surgery. *Journal of Antimicrobial and Chemotherapy*, 46(4):527-534.

Orrett, A. and Land, G (2016). Methicillin-resistant *Staphylococcus aureus* prevalence: current susceptibility patterns in Trinidad. *BMC Infectious Diseases*, **6**. 1:1–6,

Patti J. M., Tarkowski A. and Josefsson E. (2012). Expression of staphylococcal clumping factor A impedes macrophage phagocytosis. *Journal of Infectious Diseases*, 6:18–19

Peng, C.F., Chiang, Y.H. and Chen T.P. (2015). Risk factors and molecular analysis of community methicillin-resistant *Staphylococcus aureus* carriage. *Journal Clinical Microbiology, 4*3:132-139

Rao, V.I., Pai, V. and Rao, S.P. (2017) Prevalence and Antimicrobial Susceptibility Pattern of Methicillin-resistant *Staphylococcus aureus* [MRSA] Isolates at a Tertiary Care Hospital in Mangalore, South India. *Journal of Laboratory Physicians,* 2:82-84.

Robertson, G.J., Merlino, J. and Gill,R. (2010). Application of lipovitellin-salt-mannitol agar for screening, isolation, and presumptive identification of *Staphylococcus aureus* in a teaching hospital. *Journal Clinical Microbiology,* 34(12):3012-3015.

Robinson, D.A. and Enright, M.C. (2014). Multilocus sequence typing and the evolution of methicillin-resistant *Staphylococcus aureus. Clinical Micrbiology Infections*, 0:92-97.

Silver, S., Sanita P.V., Barbugli P.A., Dias, C.M., Lordello, V.B., Vergani, C.E. (2015). Dynamics of biofilm formation and the interaction between *Candida albicans,* methicillin-susceptible (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA). 14:122-131

Velasco, D., Tomas, M. and Cartelle, M. (2017). Evaluation of different methods for detecting methicillin (Methicillin) resistance in *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy,* 55: 379–382