MOLECULAR DETECTION OF METHICILLIN RESISTANCE GENES FROM CLINICAL SOURCES AND HOSPITAL ENVIRONMENTS

Abstract

Infections associated with *Staphylococcus aureus* have high mortality rate and lead to economic loss with a long stay in the hospital. Methicillin-resistant *S. aureus* (MRSA) is one of the major nosocomial pathogens which are acquired in the health care facilities. The objective of the study was to investigate the presence of MRSA in clinical sources and hospital environments. Samples were collected, cultured and identified. Also, the antibiotic susceptibility profile was done. Polymerase chain reaction (PCR) amplification of identification gene *nuc*- and the resistant gene, *mecA* were conducted. Sixty-three isolates were positive for *S. aureus* out of 370 clinical samples (urine, wound, nasal swabs and pus) and 37 positives out of 262 samples from hospital environments. Majority of the isolates were sensitive to cefoxitin, novobiocin and majority were resistant to ceftazidine, cloxacillin and augmentin. Seventy-one to ninety- five percent and 51-72% exhibited multi-drug resistance among clinical samples and hospital environments respectively. Both samples were positive for *nuc* and *mec A* genes. The detection of MRSA in hospital environments may pose a great danger to patients especially those of compromised status.

Keywords: Methicillin-resistant *Staphylococcus aureus*, clinical samples, hospital environments, multidrug resistance

INTRODUCTION

Staphylococcus aureus is a Gram-positive coccus that occurs in grape-like clusters. It is a eubacterium that is found on the surface of the human skin and mucous membranes (1-2). They are part of the normal microbial flora of the skin, intestinal and upper respiratory tract but may become pathogenic for compromised individuals whose defenses have been weakened by illness or therapy. *S. aureus* is an opportunistic organism in man and animals and is the most frequent cause of nosocomial (hospital) and community infections (3-4). It has the ability to cause a range of illness from minor skin infections such as abscesses, boils to life-threatening diseases such as meningitis, pneumonia, sepsis and toxic shock syndrome (TSS) (5).

Isolation of MRSA has been reported from a variety of environmental sites including door handles, floors, linen, gowns, tables, beds, infusion pumps, computer terminals, and ventilation grills (6-8). Contamination can be widespread in some instances. For example, Boyce *et al*., (1997) reported the isolation of MRSA from 27% of surfaces in rooms of MRSA positive patients, with the most common site being flooring.

Increasingly, nosocomial isolates are resistant to multiple drugs. In the community, *S. aureus* remains an important cause of skin and soft tissue infections, respiratory infections, and infective endocarditis (9). Scientific evidence suggests that environmental contamination plays an important role in the spread of methicillin-resistant S. *aureus*.

Therefore, this study investigated the presence of MRSA in General Hospital, Asubiaro, Osogbo, Osun State.

MATERIALS AND METHOD

Area of Study

The area of the study was Osun State General Hospital, Asubiaro and is located within the Osogbo Local Government town of Osun State in the South West Zone of Nigeria.

Collection of samples

Samples were collected using sterile cotton swabs moistened in sterile peptone water. The swab was firmly applied, slowly rotated and thoroughly covering the surface of the wound. The same was done for patient beds, staff table, door knobs, benches, floors, toilet seats, stretchers, and operation table. The urine sample was collected in a sterile universal bottle. The swab was placed in a sterile nutrient broth, placed in an ice pack, and then taken immediately to the laboratory for culturing.

Preparation of culture media

Nutrient agar, Nutrient broth, Mueller Hinton agar, Mannitol salt agar, were performed according to manufacturers' instructions

. **Inoculation and incubation**

Each sample swab was inoculated into prepared sterile bacterio-logical peptone water and incubated at 37^{o}C for 24 h for enrichment after which the turbid broth was subcultured unto solid differential media such as Mannitol salt agar, and incubated again at 37^{o}C for 24 h. Discrete colonies were further subcultured onto freshly prepared plates of the selective media and nutrient agar plates to obtain pure cultures. The purified cultures were gram stained and stored on nutrient agar slants for biochemical tests and identification.

PHENOTYPIC CHARACTERIZATION OF THE ISOLATES

Staphylococcus aureus strains were identified by yellow colony morphology, pigmentation and growth on Mannitol salt agar (Merk). They were identified on the basis of Gram staining and colonial morphology and biochemical tests such as catalase and coagulase. Obtained results were compared with specifications in Bergey'Manual of Systematic Bacteriology

GENOTYPIC IDENTIFICATION OF THE ISOLATES

DNA EXTRACTION

The DNA of the isolates was extracted by suspending 4-5 bacteria colonies in 500 µl of TBE buffer in Eppendorf tubes appropriately labeled. The cells were boiled at 100^{o}C for 10 minutes and were cooled rapidly on ice for 30 minutes. 3ul of Proteinase K was added to the lysed cells and the mixture was incubated for 15-20 minutes at 55-60^{o}C. The enzyme was denatured by boiling at 100^{o}C for 10minutes and was centrifuge at 13, 400 rpm. The supernatant containing the DNA was collected for PCR and was stored at -20^{o}C for further use.

Primers used for PCR amplification of nuc gene and mecA gene are shown in Table 1 below.

Table 1: Layout of primer used

Target	Primer	Sequence	Annealing temp. (^{0}C)	Size	Reference
nuc A	nuc F nuc R	GCGATTGATGGTGATACGGTT AGCCAAGCCTTGACGAACTAAAGC	58	276	Goudarzi *et al*., 2016
mec A	mecAF mecAR	GATCTGTACTGGGTTAATCA CATATGACGTCTATCCATTT	57	500	(Angela *et al.*, 2015)

GEL ELECTROPHORESIS

After successful amplification, PCR products were resolved on l% agarose gel prepared by dissolving 1g of agarose powder in 100 ml of 1X Tris-borate-EDTA (TBE) buffer solution inside a clean conical flask. The 1% agarose solution was heated in a microwave oven for 2-3 minutes and was observed for clarity which was an indication of complete dissolution. The mixture was then allowed to cool to about 50^{o} C after which 0.5 µl of ethidium bromide was then added. It was allowed to cool further and then poured into a tray sealed at both ends with support to form a mould with special combs placed in it to create wells. The comb was carefully removed after the gel had set and the plate was placed inside the electrophoresis tank which contained 1X TBE solution stained with 1ug/ml of ethidium bromide solution and loaded to the well of the agarose gel. The power supply was adjusted to 100 volts for 25 minutes. For each run, a 100 base-pair molecule weight DNA standard (size marker) was used to determine the size of each PCR product. The DNA bands were then visualized with a short wave ultraviolet trans-illuminator and photographed using gene gel bioimaging system. The PCR product was then analyzed.

ANTIBIOTIC SUSCEPTIBILITY TESTING

The Clinical Laboratory Standard Institute (CLSI) modified disc agar diffusion technique was used for antibiotics susceptibility test. Discrete colonies of confirmed *S. aureus* isolates growing on nutrient agar plates were emulsified in 3mL of phosphate-buffered solution and turbidity adjusted to 0.5McFarland standard. Using a sterile swab stick, the surface of Mueller Hinton agar in a 90mm diameter petri dish was inoculated with the bacterial suspension by streaking evenly the whole surface of the agar in three different directions. The inoculated plates were allowed to dry for about 10 minutes before aseptically placing the antibiotic discs to the surface of the agar. They were then allowed to dry for further 30 minutes and then incubated at 35^{o}C. The zones of inhibition produced by each antibiotic disc

were measured and the isolates were classified as resistant, intermediate, and sensitive based on the standard interpretative chart of the NCCLS and Fluka zone interpretative chart in accordance with WHO requirement. The *Staphylococcus aureus* isolates were tested for methicillin resistance using oxacillin disc of 1 µg and cefoxitin disc of 30 µg and for vancomycin resistance using vancomycin disc of 30 µg as recommended by Andrews *et al*., 2007. Susceptibility of the isolates to other antibiotics namely; ceftazidine (30 µg), cefuroxime (30 µg), ceftriaxone (30 µg), cloxacillin (5 µg), augumentin (30 µg), fusidic acid (5 µg), novobiocin (5 µg), sulphamethoxazole (25 µg), gentamicin (10 µg), ofloxacin (5 µg) and erythromycin (5 µg), was also determined as recommended by Andrews *et al*., 2007.

RESULTS

Sixty- three (63) isolates were positive for *S. aureus* out of 370 clinical samples, of which 11 were isolated from 97 urine samples*;* 21 were isolated from 74 wound samples; 15 isolated from 123 nasal swabs and16 out of 76 pus samples (Table 2) . Out of 20 staff tables 5 were positive, from 40 doorknobs 7 were positive, out of 32 beds 6 were positive, from 21 benches 3were positive, from 61 different wards' floor 12 were positive, out of 22 toilet seats 1 was positive, from 31 stretchers 3 were positive, of 35 operation tables none was positive for *S. aureus* totalling 37 *S. aureus* isolates out of 262 hospital environments (Table 3).

A total of 44 (%) of MRSA were identified out of 100 *S. aureus* (63 from clinical samples and 37 from hospital environments).Out of the 44 MRS isolates, 63.6% (28) from clinical samples and 36.4% (16) from hospital environments respectively. The majority were sensitive to cefoxitin 79% (50/63) among clinical samples and 97% (34/35) from hospital environments as shown in Table 3 and 4 respectively.

Table 2: Positive samples of *Staphylococcus aureus* among clinical isolates

Specimen	Sample Size	*S.aureus*	Percentage distribution (%)
Urine	97	11	17.46

Wound	74	21	33.33
Nasal swab	123	15	23.81
Pus	76	16	25.40
Total	370	63	100

Table 3: Percentage distribution of *Staphylococcus aureus* found in the hospital environments.

Specimen	Size	*S.aureus*	Percentage distribution (%)
Staff tables	20	5	13.51
Door knobs	40	7	18.92
Beds	32	6	16.22
Benches	21	3	8.1
Floors	61	12	32.43
Toilet seats	22	1	2.7
Stretchers	31	3	8.1
Operation tables	35	0	0
Total	262	37	100

Table 4: Antibiotic sensitivity profile of *Staphylococcus aureus* from clinical isolates.

Antibiotics	concentration(µg)	No.(%) Sensitive	No. (%) Resistance	No.(%) Intermediate
Ceftazidime	30	2 (3.17)	60 (95.23)	1 (1.59)
Cefuroxime	30	9 (14.29)	45 (71.43)	9 (14.29)
Gentamicin	10	32(50.79)	27 (42.86)	4 (6.35)
Ceftriaxone	30	9 (14.29)	34 (53.97)	20 (31.75)

Erythromycin	5	15 (23.81)	34 (53.97)	14 (22.22)
Cloxacillin	5	7 (11.11)	56 (88.89)	0
Ofloxacin	5	37 (58.73)	24 (38.09)	2 (3.2)
Augmentin	30	10 (15.87)	53 (84.13)	0
Fusidic acid	5	31 (49.21)	32 (50.79)	0
Novobiocin	5	46 (73.02)	11 (17.46)	6 (9.52)
Cefoxitin	30	50 (79.37)	8 (12.70)	5 (7.94)
Oxacillin	1	28 (44.44)	29 (46.03)	6 (9.52)
Vancomycin	30	34 (53.97)	22 (34.92)	7 (11.11)
Cotrimoxazole	25	35 (55.60)	22(34.92)	6(9.52)

Table 5: Antibiotic sensitivity profile of *Staphylococcus aureus* from the environments.

Antibiotics	Concentration(µg)	No.(%) Sensitive	No. (%) Resistance	No.(%) Intermediate
Ceftazidime	30	35 (94.59)	2 (5.41)	0
Cefuroxime	30	7 (18.92)	27 (72.97)	3 (8.11)
Gentamicin	10	22 (59.46)	14 (37.84)	1 (2.70)
Ceftriaxone	30	12 (32.43)	17 (45.95)	8 (21.62)
Erythromycin	5	11 (29.73)	19 (51.35)	7 (18.92)
Cloxacillin	5	24 (64.86)	12 (32.43)	1 (2.70)
Ofloxacin	5	29 (78.38)	6 (16.23)	2 (5.41)
Augmentin	30	16 (43.24)	21 (56.76)	0
Fusidic acid	5	22 (59.46)	15 (40.54)	0
Novobiocin	5	30 (81.08)	3 (8.11)	4 (10.81)
Cefoxitin	30	34 (91.89)	1 (2.70)	2 (5.41)

Oxacillin	1	16 (43.24)	14 (37.84)	7 (18.92)
Vancomycin	30	20 (54.05)	11 (29.73)	6 (16.23)
Cotrimoxazole	25	25 (67.57)	10 (27.03)	2 (5.41)

Amplification of confirmatory and the resistant genes

The *nuc A* and *mec A* primers used for this PCR are in (Table 1), and the process was carried out at normal conditions as described earlier. The *nuc A* identification gene was observed at 533bp (Fig 1). Resistant gene *mec A* was observed at 276bp (Fig 2).

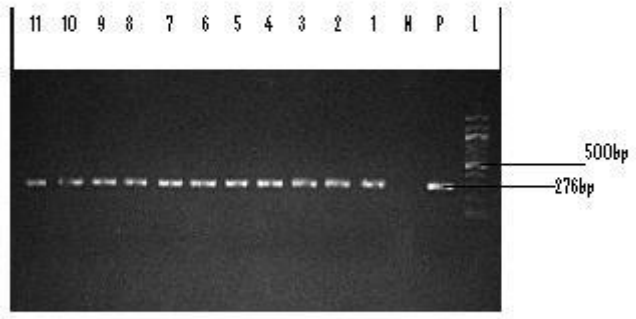

Figure 1: Agarose gel electrophoretogram of *nuc* gene Methicilin- resistant *S.aureus* after PCR analysis which bands at 276bp

Key:

L (100 bp ladder)

P –Positive

N-Negative

Lanes 1 -11 are the *nuc* positive samples of MRSA

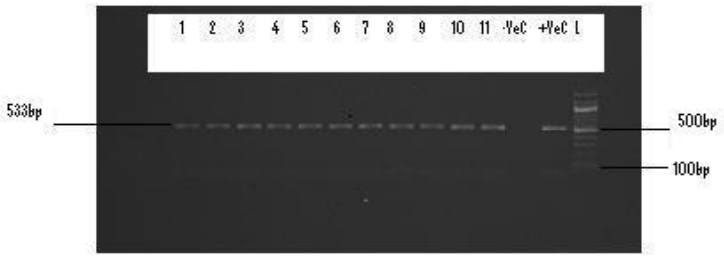

Figure 2: Agarose gel electrophoretogram of *mecA* Methicilin-resistant *S. aureus* after PCR analysis which bands at 500 bp

Key:

L -100 bp ladder (DNA marker fragments)

Pos – The MRSA positive control

Neg-Negative

Lanes 1 -11 are the *mecA* positive samples

Seventy six percent (76/100) of the *S. aureus* isolates were positive for *nuc* gene and thus were categorized as strains of *S. aureus*. All isolates of MRSA were positive for *nuc* gene. However, 25% (11/44) of the MRSA were positive for *mec A* gene.

Discussions

Hospital is a major reservoir of variety of pathogens. These pathogens are usually normal flora of the human body especially *S. aureus*, which is known to cause nosocomial infection. MRSA strains are often resistant to a wide range of antimicrobials, thereby posing a great danger to those are infected. Especially, those of broken skin or mucous membrane and people with suppressed immune system. Previous hospitalisation, length of hospitalisation, prior and prolonged antibiotic treatment and presence of invasive indwelling devices may be risk factors for MRSA colonisation and infection. Furthermore, the isolation rate was highest from wound discharges (33.3%), followed by pus (25.4%) and nasal swab and urine samples had

isolation rates of 23.8% and 17.5%, respectively. Also, the isolation rate was highest in the hospital floor (32.4%), followed by door knobs (18.9%) and none was isolated from operation tables. The higher frequency of *S. aureus* isolation in the wound was also observed by Udobi *et al*., (10). The prevalence of MRSA in clinical sources and hospital environment is very high (44%) when compared with previous report of Gorwitz and colleagues (11) which was in the range of 21%–30% though it is similar to what was gotten in Jos (43%) (13).The methicillin disc for MRSA detection in these studies might have been responsible for the higher prevalence recorded in them(14-15). That is through the cultural method, the study revealed that 44% (44/100) of *S. aureus* were tested positive for MRSA. However, when polymerase chain reaction (PCR) for *mecA* detection was done 11 out of 44 were positive. This may be due to hyperproducing penicillinase strains of *S. aureus* which gave false-positive result for MRSA (16).Resistance to oxacillin was 46.03% and 37.84% among clinical isolates and hospital environments respectively. This is in contrast to an Iranian study, where resistance was 88% among clinical samples. Oxacillin and methicilin are hardly used in our setting as compared with the Iranian study which noted the rampant use of oxacillin. Our report is however similar to observations by Olowe *et al.* and Terry *et al*. in Osogbo, Nigeria, where 40.4% of *S. aureus* clinical isolates were resistant to oxacillin (17-18). The study also shows that majority of *staphylococcal* isolates were multidrug resistant, seventy-one to ninety- five percent and 51-72% exhibited multi-drug resistance among clinical samples and hospital environments respectively. The finding is similar to an observation by Akinjogunla and Enabulele (19).

Recommendations

MRSA may be reduced by aseptic techniques for all procedures and processes, careful handling of contaminated materials, continuous sterilisation of hospital floors, effective screening of patients, proper hand-washing technique, compliance to antimicrobials topical agents named Mupirocin to be widely used, body cleansing agents such as (povidone, triclosan) can be used, isolation of patients with MRSA can also be done.

Ethics Statement

This work was performed according to University ethics committee code of conduct.

REFERENCES

1. Dilnessa T, Bitew A. 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, (2016) vol. 16, no. 1, p. 398. View at Publisher · View at Google Scholar · View at Scopus

2. Gonsu KH, Kouemo SL, Toukam M, Ndze VN, Koulla SS. Nasal carriage of methicillin resistant Staphylococcus aureus and its antibiotic susceptibility pattern in adult hospitalized patients and medical staff in some hospitals in Cameroon," Journal of Microbiology and Antimicrobials, (2013) vol. 5, no. 3, pp. 29–33,. View at Publisher · View at Google Scholar

3. Deyno S, Fekadu S, Astatkie A. Resistance of Staphylococcus aureus to antimicrobial agents in Ethiopia: a meta-analysis," Antimicrobial Resistance and Infection Control, (2017) vol. 6, no. 1, p. 85. View at Publisher · View at Google Scholar · View at Scopus

4. Omuse G, Kariuki S, Revathi G. Unexpected absence of methicillin-resistant Staphylococcus aureus nasal carriage by healthcare workers in a tertiary hospital in Kenya," Journal of Hospital Infection, (2012) vol. 80, no. 1, pp. 71–73. View at Publisher · View at Google Scholar · View at Scopus

5. Ojulong J, Mwambu T, Jolobo M. Prevalence of methicillin resistant Staphylococcus aureus (MRSA) among isolates from surgical site infections in Mulago hospital-Kampala, Uganda," Internet Journal of Infectious Diseases, (2008) vol. 7, no. 2, p. 10326,. View at Publisher · View at Google Scholar

6. Meyer E, Schro¨der C, Gastmeier P, Geffers C. The reduction of nosocomial MRSA infection in Ger-many:an analysis of data from the Hospital Infection Surveillance System(KISS)between2007and2012.DtschArzteblInt.2014;111(19):3316.doi:10.3238/arztebl.2014.0331PMID:24875457

7. Geffers C, Gastmeier P. Nosocomial infectionsandmultidrug-resistantorganisms in Germany: epide-miological datafromKISS(theHospital InfectionSurveillance

System).DtschArzteblInt.2011;108(6):87–
93.doi:10.3238/arztebl.2011.0087PMID:213732754.

8. Dulon M, Peters C, Schablon A, NienhausA.MRSAcarriageamonghealthcare workersin nonoutbreaksettingsinEuropeandtheUnitedStates:asystematicreview.BMCInfectDis.2014;14(1):363.

9. Centers for Disease Control and Prevention.. Active bacterial core surveillance (ABCs) report, emerging infections program network, methicillin-resistant Staphylococcus aureus, 2012. http://www.cdc.gov/abcs/reports-findings/survreports/mrsa12.pdf

10. Udobi CE, Obajuluwa AF, Onaolapo JA . "Prevalence and antibiotic resistance pattern of methicillin-resistant *Staphylococcus aureus* from an orthopaedic hospital in Nigeria," BioMed Research International, vol. 2013, Article ID 860467, 4 pages, 2013. View at Publisher · View at Google Scholar · View at Scopus

11. Gorwitz J, Jernigan DB, Powers JH, Jernigan JA. Participants in the CDC Convened Experts' Meeting on Management of MRSA in the Community. Strategies for Clinical Management of MRSA in the Community: Summary of Experts Meeting Convenes by the Centre for Disease Control and Prevention; March, 2006.

12. Nasiru A, Kenneth CI. Methicillin-Resistant *Staphylococcus aureus* in a Central Nigeria Tertiary Hospital Annals of Tropical Pathology 2018 vol. 9, 6-10

13. Ikeh EI. Methicillin-resistant *Staphylococcus aureus* (MRSA) at Jos teaching hospital. Afr J Cli Exp Microbiol 2003; 4: 52-5

14. Taiwo SS, Onile BA, Akanbi AA. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in Nigeria. Afr J Clin Exp Microbiol 2004; 5:189-97.

15. Onemu OS, Ophori EA. Prevalence of multi-drug resistant *Staphylococcus aureus* in clinical specimens obtained from patients attending the university of Benin teaching Hospital, Benin City, Nigeria. J Nat Sci Res 2013; 3:154-9.

16. Memmi, G, Filipe, SR., Pinho, MG, Fu Z, Cheung, A. Staphylococcus aureus PBP4 is essential for beta-lactam resistance in community-acquired methicillin-resistant strains. Antimicrob Agents Chemother, (2008) 52, 3955-66

17. Olowe OA, Kukoyi OO, Taiwo SS, Ojurongbe O, Opaleye OO, Bolaji OS. Phenotypic and molecular characteristics of methicillin-resistant *Staphylococcus aureus* isolates from Ekiti state, Nigeria. Infect Drug Resist 2013; 6:87-92

18. Terry OA, Ogbolu DO, Akorede E, Onemu OM, Okanlawon BM. Distribution of mecA gene amongst *Staphylococcus aureus* isolates from south western Nigeria. Afr J Biomed Res 2011; 14:9-16

19. Akinjogunla OJ, Enabulele IO. Virulence Factors, Plasmid Profiling and Curing analysis of Multidrug Resistant *Staphylococcus aureus* and Coagulase negative *Staphylococcus* species isolated from Patients with Acute Otitis Media. Journal of American Science. 2010; 6(11):1022–1033.