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Original Research Article

Isolation and molecular identification of methicillin resistant *staphylococcus aureus* (MRSA) from different type of wound (Cuts, burn and chemical)

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ABSTRACT

Three hundred of clinical wound samples (Cuts, burn and chemical) were collected from Murtala Muhammad Specialist Hospital, Kano State. The isolation and identification of *Staphylococcus aureus* was done through culture on Nutrient agar and Mannitol salt agar, Gram staining, microscopic and standard biochemical tests such as (catalase, coagulase, oxidase and hemolysis test) were carried out. Cefoxitin disc diffusion test and molecular analysis was done for the detection of methicillin resistant strain of *S. aureus*. A total of one hundred and one *Staphylococcus aureus* isolates were identified in the present study in which 13(12.90%) were MRSA positive and 88(87.10%) were negative MRSA. Polymerase chain reaction (PCR) analysis reveals the presence of mec A gene under 310base pairs nucleotide sequence in the positive MRSA.

1. Introduction

Resistance to methicillin that indicated resistance to all beta-lactam agents was first reported in 1961, which marked the appearance of methicillin-resistant *Staphylococcus aureus* (MRSA).[1] Methicillin resistant *Staphylococcus aureus* (MRSA) is a strain of *Staphylococcus aureus* that is resistant to antibacterial activity of methicillin and other related antibiotics of the penicillin class and it belongs to the large group of bacteria known as Staphylococcus often referred to as Staph infection, MRSA are most common in hospital and other institutional healthcare settings, thereby many researchers are aiming to scientifically prove the used of *Medicinal plant* as an effective means of controlling antibiotics resistance.[2]

2. Aim

The main of the study is to identify the methicillin resistance among the isolates of *Staphylococcus aureus* from different type of wound.

2.1. Specific objectives of the study

1. To isolate and identify *Staphylococcus aureus* from clinical specimens of wound.
2. To Detect methicillin-resistant Strains using Cefoxitin disc diffusion.
3. Molecular analysis.

3. Materials and Methods

3.1. Study area

The study was conducted in Murtala Muhammad Specialists Hospital (MMSH), Muhammad Abdullahi Wase Road, Kano municipal, Kano State. It was the largest state government hospital in northern Nigeria and has the

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highest patients' attendance in the region. The hospital was commissioned in 1926, presently with an official bed capacity of 826 and has total number of 30 wards and units and 9 operating theatre and 14 clinics. It was also has the staff strength of 1656 and it is an NHIS accredited hospital. An ethical approval was obtained from Murtala Muhammad Specialist Hospital (MMSH), Kano state based on the consent of the Hospital Ethical Committee, under the consideration of Ministry of Health Kano.

3.2. Sample size

The sample size was calculated using 28% prevalence of MRSA in Kano.[3] The following formula was used to calculate the sample size as described by.[4]

$N = Z^2P (1-P) / d$

N=300

3.3. Inclusion criteria

All consented patients admitted in the surgical ward with cuts, burn and chemical wound for at least 14 days were included in the study.

3.4. Collection of clinical wound samples

Samples (Burn, cuts and chemical wound) were collected among the inpatients in Surgery Departmen at the 14 days interval of collection in Murtala Muhammad Specialist Hospital (MMSH), Kano State using swab technique as described by.[5]

3.5. Isolation and Identification of staphylococcus aureus

Five milliter of prepared nutrient broth was added to test tubes and sterilized by autoclaved at 121^0C for 15 minutes then allowed to cool. Swabbed sample was inoculated into sterilized 5ml of nutrient broth and uninoculated test tube was served as control. The test tubes were then incubated at 37^0C for 24 hours. After the incubation, sterile swab stick was dipped into each test tube with reference turbidity and swabbed gently on to the prepared nutrient agar (NA) plate and incubated at 37^0 for 24 hours. After 24hours of incubation, the examined colonies on a nutrient agar (NA) plate was then picked and inoculated on to the prepared mannitol salt agar (MSA) plate using sterile wire loop and incubated at 37^0 for 24-48hours. Typical Staphylococcus colonies were examined after two days of the incubation.[6,7]

3.6. Gram stain

Gram stain was achieved through the standard procedure as described by[8]

Table 1: Biochemical tests

Test	Procedure
Catalase (slide method)	9
Oxidase	10
Hemolysis (blood agar test)	11
Coagulase (slide method)	12

3.7. Standardization of the bacterial inoculums

A loopful of bacterial colony of the test isolates was picked and inoculated into test tube containing 5ml of distilled water using sterile wire loop**,** turbidity was adjusted to equivalent 0.5 McFarland standards.

3.8. Detection of methicillin-resistant staphylococcus aureus using cefoxitin disc diffusion

All *Staphylococcus aureus* isolates were subjected to cefoxitin disc diffusion test using 30ug of cefoxitin (Oxoid CT0019B). Prepared Mueller-Hinton agar plates were swabbed with standardized bacterial inoculums. Thirty microgram of cefoxitin disc were placed on the centre of MHA plates using sterile forceps and incubated at 37 ^{0}C for 24 hours. Positive methicillin resistant strain of *Staphylococcus aureus* and methicillin susceptible strain of *Staphylococcus aureus* were served as positive and negative plates control respectively obtained from Murtala Muhammad specialist hospital, Kano. Zone of diameter inhibition was measured.[13,14]

3.9. DNA extraction

Pure colonies of *Staphylococcus aureus* from those that were cefoxitin resistance were cultured in nutrient broth for 24 hours at 37^oC with frequent agitation using micro centrifuge at 100rpm for 5minutes. Cell suspension were transferred into another test tube and centrifuged at 4500rpm for 5minutes. DNA was extracted using boiling lysis method, in which 1g of cell pellets were resuspended in 40ul of water and boiled at 100^0C in water bath for 10minutes. After it was cooled on ice, and then centrifuged at 15000 x g for 10seconds. Cold 95% of ethanol was added to the supernatant fluid and stored at -20^0C until polymerases chain reaction assay (PCR).[15]

3.10. Polymerase chain reaction (PCR) assay

PCR mixture was prepared in thin walled 0.2ml tube, which contains 50ul of distilled water; Buffer 1x, $MgCl_2$ 0.5mM, Taq polymerase 0.05Units/ul, dNTP 200uM and mecA primer 0.5uM (forward) and 0.5uM (Reverse). 10ul of DNA sample was added to 50ul of PCR mixture, the mixture was placed inside the Bio-Rad thermal cycler for PCR amplification. Initial denaturation was carried out at 92^0C for 30minutes followed denaturation at 92^0C for 10minutes,

annealing at 56^0C for 10minutes and extension at 72^0C for 10minutes. The final extension was performed at 72^0c for 30minutes; the amplified product was detected by 1% of agarose gel electrophoresis with (0.5ug/ml) ethidium bromide staining and was observed using Ultraviolet light.[16]

Table 2: Oligonucleotide sequence of PCR primers employed for the identification ofmecA gene.

Name of primers	Sequence 5'-3'	Product size bp
MecA-F	GTAGAAATGACTGAACGTCCGATTA	310bp
MecA-R	CGAATTCGACATTGTTTCCGTCTAA	310bp

Sources:[17]

4. Results

4.1. Occurrences of Methicillin-resistant Staphylococcus aureus (MRSA) from different type of wound

Occurrence of *S.aureus* and MRSA were presented in table 1 below; out of 100 samples from each sites of wound (cuts, burn and chemical) has positive isolates of 31(3), 56(8) and 24(2) respectively.

Table 3: Occurrences of Methicillin-resistant *Staphylococcus* aureus (MRSA) from different type of wound

Sites of wound	*S.aureus*	Pos. MRSA	Neg. MRSA	Samples
Cuts	31	3	28	100
Burn	56	8	48	100
Chemical	24	2	22	100
Total	101	13	88	300

Key: pos. positive

4.2. Percentage occurrences' of Methicillin-resistant Staphylococcus aureus (MRSA)

Percentage occurrence of MRSA was presented in table 2 below, in which out of the 101 total number of *Staphylococcus* aureus isolates, 13(12.90%) isolates were positive MRSA and 88(87.10%) were negative MRSA.

Table 4: Percentage occurrences' of methicillin-resistant *staphylococcus* aureus (MRSA)

Isolates	Number of occurrence	Percentages (%)
Positive MRSA	13	12.90
Negative MRSA	88	87.10
Total *S.aureus*	101	100

5. Discussion

Out of the total number of 101 isolates of *Staphylococcus* aureus obtained from 300 clinical samples of cuts, burn and chemical wound based on morphological and biochemical characterization, 13(12.90%) were methicillin resistant *Staphylococcus* aureus (MRSA) and 88(87.10%) isolates were negative MRSA. MRSA were determined by corresponded standard zone of inhibition given by.[14] MRSA represented 12.90% which was evaluated in the present study from cuts, burn and chemical wound was below the findings of Mounir in his study of phenotypic and genotypic characterization of nasocomial isolates of *S.aureus* with reference to methicillin resistant who reported out of total 98 isolates of *S.aureus* from only burn wound were 24.1% positive MRSA. The difference between 12.9% of my present study and 24.1% might be due the highest number of *Staphylococcus* aureus obtained by Mounir in his study.[18] Polymerase chain reaction (PCR) analysis reveals the presence of mec A gene under 310base pairs nucleotide sequence in the positive MRSA, this present finding has similar to the previous findings by Garcia alvarez, et al who reported a novel allele of mec A gene encoding an alternative penecillin binding protien that mediate methicillin resistance among bovine *S.aureus*.[19] The mec A gene is highly conserved among Staphylococcal species that show resistance to methicillin and consequently the detection of this gene was by polymerase chain reaction machine.[20,21]

6. Conclusion

Conclusively, the study reaveled the presence of methicillin resistance strains in both kind of wound isolates (Cuts, burn and chemical) but with highest occurrence in burn wound.

7. Recommendation

MRSA is very serious infection that need to take all the necessary measures, the control strategies should be the use of superior Antibiotics and other physical measures which includes properly drained, cleaned and dressed purulent exudates, hand washing, discourage sharing personal items, used proper wound covering and proper sanitization of reusable items in the hospital and homes.

8. Source of Funding

None.

9. Conflicts of interest

There are no conflicts of interest.

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