MODERN APPLIED MEDICAL RESEARCH (MAMR)

1(2): 33-37
ISSN: 2582-9181

ISOLATION, CHARACTERIZATION AND DETERMINATION OF PREVALENCE RATE OF METHICILIN RESISTANCE *STAPHYLOCOCCUS AUREUS* (MRSA) FROM DIFFERENT TYPES OF WOUND

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REVIEW ARTICLE

Received: **29-01-2021**
Accepted: **03-02-2021**
Published: **05-02-2021**

Abstract: The study was aimed to isolate, characterize and determine the prevalence of Methicillin resistance *Staphylococcus aureus* (MRSA) from different types of wound. A total of eighty (80) samples from different categories of wound were collected from Muhammad Abdullahi Wase Hospital Kano over a period of six months (March, 2016 to August, 2016). The isolation of *Staphylococcus* isolates was done by culturing the various clinical samples of infected wounds (n=41), sepsis wound (n=19), bite wound (n=12) and surgical wound (n=8) on a surface of freshly prepared Nutrient agar. Each colony was isolated in a pure form by sub culturing for further studies and identification. Identification of the isolates was conducted using Gram staining, biochemical test and microbiological analysis of the isolates. The result showed that the MRSA were positive for Gram staining, catalase, coagulase, DNase and were able to ferment Mannitol. The isolates showed β-haemolysis on blood agar plates and resistance to both oxacillin and cefoxitin. From the result, 15% of the *staphylococcus* species isolated were MRSA and highest prevalence was found among infected wound (7.5%). Statistical analysis of the result showed significant difference in the prevalence of *Staphylococcus* species among the wound samples examined at $p<0.05$. It is concluded that *Staphylococcus* species are one of the etiological agents of wound infection.

Keywords: *Characterization, isolation, Kano, prevalence, Staphylococcus aureus*

Introduction

Wound infection causes great distress in terms of associated mortality and morbidity, increased length of hospital stays, profound discomfort and significant increase in healthcare cost. Infection in a wound delays healing and may cause wound break down, herniation of the wound and complete wound dehiscence [1]. Therefore, the knowledge of the causative agents of wound infection will assist in the control and prevention of such infection and also help in selecting empirical antimicrobial therapy for the control of causative microorganisms [2]. Some aerobic pathogenic organisms such *P. aeruginosa*, *S. aureus* and beta haemolytic *Streptococci* have been most frequently reported as the cause of delay wound healing [2,3].

Staphylococcus aureus is an opportunistic pathogen often carried asymptomatically on the human body [4]. *Staphylococci* are group of bacteria frequently isolated as etiologic agents of various infectious diseases with *Staphylococcus aureus* being the most important human pathogen [5]. S. aureus has long been recognized as one of the most important bacteria that cause disease in humans. It is the leading cause of skin and soft tissue infections such as abscesses (boils), furuncles and cellulitis. Although most Staphylococcal infections are not serious, *S. aureus* can cause serious infections such as blood stream infections, pneumonia, or bone and joint infections [6]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of *Staphylococcus aureus* that is resistant to the antibacterial activity of methicillin and other related antibiotics of the penicillin class [7]. It belongs to the large group of bacteria known as *Staphylococci*, often referred to as Staph. About 25%-30% of all people have Staph within the nose, but it normally does not cause an infection. In contrast, only about 1% of the populations have MRSA [8]. Infections with MRSA are most common in hospitals and other institutional health-care settings, such as nursing homes, where they tend to affect older people, those who are very ill, and people with a weakened immune system [9]. Methicillin resistant *Staphylococcus aureus* is especially troublesome in hospitals, prisons, and nursing home, where patients with open wounds, invasive devices and weakened immune systems are at greater risk of nosocomial infection than the general acquired infection, but has developed limited endemic status and is now sometimes community acquired [10]. *Staphylococcus aureus* most commonly colonized under the anterior (the nostril) the rest of the respiratory tract, open wound, intravenous catheters, and the urinary tract also potential site for infection [10]. The study was aimed to isolate, characterize and determine the prevalence rate of *Staphylococcus* species from different types of wound.

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Published online at http://gulfpublishers.com/

Materials and Methods

Study Area

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ISSN: 2582-9181

The study area is Kano metropolis, samples from infected wound patients were collected from Muhammad Abdullahi Wase Hospital in the state capital. Kano State is located in the North-west Nigeria located at latitude 11^0 30 N and longitude 8^0 30 E. It share borders with Kaduna state to the south- west, Bauchi state to the South-East, Jigawa state to the East, Katsina state to the North [11]. It has a total area of 20,131km^2 (7,777sqm) and estimated population of 13.4 million [12].

Ethical approval

Ethical approval (Issue number: HMB/ GEN/488/Vol. I) was obtained from Health Service Management board (HSMB) Kano State based on the consent of Muhammad Abdullahi Wase Specialists Hospital (MAWSH) Ethical Committees.

Bacterial Isolation Sample collection

A total of eighty (80) samples from different categories of wound were collected from Muhammad Abdullahi Wase Hospital Kano over a period of six months (March, 2016 to August, 2016). The isolation of *Staphylococcus* isolates was done by culturing the various clinical samples of infected wounds (n=41), sepsis wound (n=19), bite wound (n=12) and surgical wound (n=8) on a surface of freshly prepared Nutrient agar (Lifesave Biotech, USA). The plates were incubated at 37^0C for 24 hours for colony formation [13]. Each colony was isolated in a pure form by sub culturing for further studies and identification. Discrete colonies were kept in peptone water. The bacterial strains were then stored at 4^0C for further use.

Identification of bacteria

The isolates were subjected to Gram staining, Biochemical tests (Catalase, coagulase, DNase, haemolysis test) and Mannitol fermentation and as described by Holt *et al.* [14] and Cheesbrough [13] for identification.

Gram staining

A drop of normal saline was placed on a well labeled clean grease-free glass slide using a sterile inoculating loop; a colony of an overnight culture of the bacterial isolate was emulsified with the normal saline to make a thin smear. The smear was air dried and then heat fixed. The slide was flooded with crystal violet (primary stain) for 30 seconds after which the stain was rinsed from the slide with water. The smear was flooded with Lugol's iodine (mordant) to fix the primary stain. The iodine was rinsed with water after 60 seconds**.** The slide was then flooded with a decolorizer (acetone) and rinsed off almost immediately**.** The counter stain; safranin was added and left for 30 seconds before being rinsed off. The stained smear was air dried, and then observed under the microscope using X100 oil immersion objective lens of the microscope [13].

Biochemical characterization of the Isolates

DNase test

An overnight broth culture of the organisms was spot inoculated onto agar surfaces of the DNase agar and incubated at 37^0C for 24 hours. At the end of the incubation period, the agar surface was flooded with 1N hydrochloric acid and excess drained off [13].

Catalase test

A drop of 3% hydrogen peroxide was placed on a clean grease-free glass slide. A colony of the bacteria was picked from a culture plate using a sterile wire loop and placed on the hydrogen peroxide; presence of bubbles observed indicated a positive Catalase test [14].

Coagulase test

About two drops of blood plasma were placed on a clean grease-free glass slide and a colony of the organism was picked using a sterile wire loop from an incubated nutrient agar plate. The colony was emulsified in the blood plasma and observation of a clot indicated a positive Coagulase test [14].

Mannitol test

The pure colonies on nutrient agar were picked using a sterile inoculating loop and sub-cultured onto the surface of Mannitol Salt Agar (Lifesave Biotech, USA). Then, the plates were incubated at 37^0C for 24 hours. The changes in color of the medium from pink to golden yellow indicated positive results [13].

Identification of Methicillin Resistant *Staphylococcus aureus*

All the confirmed isolates of *S. aureus* (n=67) were tested by oxacillin disc diffusion, cefoxitin disc diffusion and oxacillin screen agar test for detection of Methicillin Resistant *Staphylococcus aureus* (MRSA).

Oxacillin and cefoxitin disc diffusion method

The bacteria suspension adjusted to 0.5 McFarland were subjected to antibiotic susceptibility testing using agar disc diffusion method as described by Bauer *et al*. [15]. Mueller Hinton agar (MHA) plates were inoculated with overnight culture of each isolate by streak plating. The 1 µg oxacillin sensitivity discs (Hi-Media) and 30 µg cefoxitin sensitivity discs (Hi-Media) were then aseptically placed at equidistance on the plates and allowed to stand for 1 hour. The plates were then incubated at 37°C for 24 hours. Sensitivity pattern of the isolates to the discs based on zones of produced. Zone of inhibition was interpreted according to CLSI [16] criteria: susceptible, >13 mm; intermediate, 11–12 mm; and resistant <10 mm [17]. A commercially prepared antibiotic disc containing 10 µg clindamycin was used as positive control.

Oxacillin screen agar test.

A bacterial inoculum of each strain was made and turbidity was adjusted to 0.5 McFarland. One drop of this suspension was inoculated on Mueller–Hinton agar containing 4% NaCl and 6 mg oxacillin per ml (Hi-Media). Plates were incubated at 37^0C for 24 hours. Any strain showing growth on the plate containing oxacillin was considered to be resistant to methicillin [17]

Result

Frequency and Prevalence of wound categories

The frequency and prevalence of different categories of wound used in the study is presented in Table 1. The table showed that infected wound has the highest frequency (41) which accounted for 51.25% of the total samples used. This is followed by sepsis (19) 23.75%, bite wound (12) 15% and least frequency was recorded by surgical wound (08) 10%.

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Table 1: Frequency and Prevalence of different types of wound samples used

S/N	Types of wound	Frequency (n)	Prevalence (%)
1	Infected wounds	41	51.25
2	Sepsis wound	19	23.75
3	Bite wound	12	15.00
4	Surgical wound	08	10.00
	Total	**80**	**100**

Isolation of *Staphylococcus* species

The frequency and prevalence of different categories of *Staphylococcus* species isolated from different types of wound is presented in Table 2. The table showed that infected wound has the highest frequency (37) which accounted for 46.25% of the total samples used. This is followed by sepsis (12) 15%, bite wound (11) 13.75% and least frequency was recorded by surgical wound (07) 8.75%.

Table 2: Isolation of *Staphylococcus* species from different types of wound

S/N	Types of wound	Frequency (n)	Staph isolates (n)	Prevalence (%)
1	Infected wounds	41	37	46.25
2	Sepsis wound	19	12	15.00
3	Bite wound	12	11	13.75
4	Surgical wound	08	07	08.75
	Total	**80**	**67**	**83.75**

Identification of *Staphylococcus* species

Table 3 showed the result of morphological and biochemical characterization of *Staphylococcus* species. *S. aureus* and MRSA are positive to catalase, coagulase, and DNase tests. However, MRSA are positive to oxacillin sensitivity testing. Coagulase negative *Staphylococcus aureus* are negative to coagulase, and DNase tests.

Table 3: Identification of *Staphylococcus* species

Isolate code	GS	CA	CO	DN	MF	HAE	OST	Suspected isolates
S_1	+	+	+	+	+	+	-	*Staphylococcus aureus*
S_2	+	+	+	+	+	+	+	MRSA
S_3	+	+	-	-	-	-	-	CoNS

Key: + = positive, - = negative, GS= Gram staining, CA= Catalase, CO= Coagulase, HAE= Haemolysis, MF= Mannitol Fermentation, OST= Oxacillin sensitivity test, MRSA= Methicillin Resistance *Staphylococcus aureus*, CoNS= Coagulase Negative *Staphylococcus aureus*

Prevalence of Methicillin Resistance *Staphylococcus aureus* (MRSA)

The frequency and prevalence of MRSA from *Staphylococcus* species isolated from different types of wound is presented in Table 4. The table showed that a total of 10 MRSA were isolated from *Staphylococcus* species which accounted for 15%. Infected wound has the highest number of MRSA isolates (5) which accounted for 7.5%. This is followed by sepsis and surgical wounds (2 each) 3% and least frequency was recorded by bite wound (01) 1.5%.

Table 4: Prevalence of Methicillin Resistance *Staphylococcus aureus* (MRSA)

Types of wound	Sample (n)	Staph isolates (n)	MRSA (n)	Prevalence (%)	P-value
Infected	41	37	5	7.5	0.807622[a]
Sepsis	19	12	2	3.0	
Bite	12	11	1	1.5	
Surgical	08	07	2	3.0	
Total	**80**	**67**	**10**	**15**	

Key: [a] = the chi square value is 0.9737 and the result is not significant at $p<0.05$

Discussion

Staphylococci are frequently isolated as etiologic agents of infectious processes, with *Staphylococcus aureus* being the most important human pathogen. *S. aureus* causes superficial and deep skin and soft tissue infections, bacteremia with metastatic Staphylococci are frequently isolated as etiologic agents of infectious processes, with *Staphylococcus aureus* being the abscess formation, and a variety of toxin-mediated diseases, including gastroenteritis, staphylococcal scalded skin syndrome, and toxic shock syndrome [18]

The study aimed at isolation, identification and determination the prevalence of Methicillin resistance *Staphylococcus aureus* (MRSA) from clinical samples of different types of wound. Identification of *Staphylococcus* species in the present study was based on Gram staining, cultural characteristics and biochemical characterization. Earlier findings by Ali et al.[19]; Amengialue et al. [20]; Yabaya et al. [21]; Jahan et al. [22] identified and characterized *Staphylococcus aureus* on the basis of cultural characteristics, Gram staining and Biochemical characterization. Several studies were conducted on isolation of *Staphylococcus* species [5,18,19]. From the results obtained *S. aureus* were able to ferment Mannitol producing yellow colony, they also showed β-haemolysis on blood agar medium enriched with 5% sheep blood. Gram staining of the isolates exhibited a cluster of Gram positive cocci. The isolates were positive for catalase, coagulase and DNase test. This result was in conformity with the findings of [18,19]. In catalase test, hydrogen peroxide was broken down into water and oxygen by enzyme catalase. The production of oxygen was indicated by bubble formation [22]. The positive result of coagulase test was confirmed by the formation of curd like clotting compared to negative control [23]. From the study, the coagulase negative

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Staphylococcus (CoNS) showed negative for both haemolysis and Mannitol fermentation. This finding was in consistent with that of Ibrahim et al. [18] and that of Nwoire et al. [24] who both record the presence of coagulase negative *Staphylococcus* among clinical samples of wound in Kano and Abakaliki respectively. The non-coagulase Staphylococci identified amongst these samples might have been contaminants or opportunistic pathogens. It is well known that other Staphylococci though normal commensal are opportunistic human pathogens [25].

From the finding of this study, 15% of the *staphylococcus* species isolated were MRSA and highest prevalence was found among infected wound (7.5%). This finding was in conformity with that of Nas et al. [4] who found higher number of MRSA in wound sample. Higher number of Methicillin Resistance *Staphylococcus aureus* (MRSA) in infected wound is due to the fact that the organisms colonize human skin tissue. A gene known as *mec*A gene is responsible for the resistance to methicillin which codes for penicillin-binding protein PBP 2A [26]. The wide spread use of antibiotic resulted in the development of resistance to antibiotics through acquisition of the mobile cassette chromosome carrying the methicillin-resistant gene mecA [26] and mecC [27]. The resistance to methicillin was due to a penicillin-binding protein coded for by a mobile genetic element termed the methicillin-resistance gene –mecA [27]. In recent years, the gene has continued to evolve so that many MRSA strains are currently resistant to several different antibiotics such as penicillin, oxacillin and amoxicillin [28].

Conclusion

Finding from this study indicated the presence of *staphylococcus* species in different types of wound samples. The distribution of *Staphylococcus* species in the clinical wound samples showed that infected wound has the highest number of isolates and 15% of the *staphylococcus* species isolated were MRSA and highest prevalence was found among infected wound (7.5%). It is recommended that individual should practice good personal hygiene to avoid the spread of infection with *Staphylococcus* species

Acknowledgement

The authors wish to acknowledge to the laboratory staff of Muhammad Abdullahi Wase Specialist Hospital Kano for sample provision and use of laboratory facilities. Thanks to Kano State Government through Ministry of Health for the ethical approval.

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