IOSR Journal Of Pharmacy
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
www.iosrphr.org Volume 5, Issue 2 (February 2015), PP. 07-12

Antibiotic resistance patterns of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from chronic skin ulcer of patients in Kaduna state, Nigeria.

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ABSTRACT: *The aim of this study is to investigate the antibiotic resistance patterns of methicillin-resistant Staphylococcus aureus (MRSA) isolated from out - patients with chronic skin ulcer in four different hospitals in Kaduna state. A total number of 292 swab samples were collected from January 2012 to January 2013 and analyzed. MRSA isolates recovered were 14 (78 %) of the total number of Staphylococcus aureus found on the different sites of infection sampled. The highest number of MRSA isolates of 08 (57 %) were recovered from the leg (lower extremity ulcer), while 02 (14 %) isolates each were recovered from hand, abdominal region and mouth (buccal cavity) sites of infection. No MRSA isolates was recovered from the other parts of the skin. From the susceptibility studies of the isolates, it was found that all the MRSA isolates showed 100 % resistance to Amoxacillin + clavulanic acid (Augmentin), and least resistance of 07% to gentamicin. The antibiotic resistance pattern common to all the MRSA isolates is in the order of cefoxitin, amoxicillin + clavulanic acid, Trimethoprim + sulphamethoxazole.*

Keywords: *Antibiotic, resistance, methicillin, Staphylococcus aureus, ulcer*

I. INTRODUCTION

A wound can be defined as any injury that damage the skin and therefore compromise its protective function. However, a wound is said to be chronic when healing is not achieved within three months (Siddiqui and Bernstein, 2010). Ulcer appears to be the commonest type of chronic wound. Every open skin wounds are colonized by bacteria, this does not mean that all wounds are infected. Many factors determine the progression of a wound from contamination to infection, which may include bacterial load, the types of bacteria present in such wounds, their synergistic effect including their virulent nature (Edwards and Harding, 2004; Siddiqui and Bernstein, 2010). The initial colonizers of the skin are those bacteria that live symbiotically on the skin. The non-healing condition of a wound over time exposes it to different pathogenic bacteria.

Ulcerations of the lower leg of venous and diabetic origin are the most frequent of all chronic wounds. The delays of these wounds could be the disturbance in the supply of nutrients and removal of metabolic products, caused by the pathology of blood vessels (Bowler, 1998). Generally, infection may be caused by pathogenic bacteria originating from the external environment, as well as bacteria forming physiological microflora of the skin (Schmidt *et al*., 2000).

Staphylococcus aureus has been reported to be a major cause of several infection that includes, bacteremia, skin and soft tissue infections and osteomyelitis (Diekema *et al.*, 2001; Alam *et al.*, 2002). The root of most pyogenic local and systemic infections in both hospitals and community has been linked to *Staphylococcus aureus* (Arunara *et al*., 2013). Methicillin resistant *S. aureus* (MRSA) is often acquired when there is an individual exposure to hospitals, and other health care facilities, the consequence of this is a different serious healthcare- associated infections (Guidelines, 2008). MRSA isolates that were first recognized in the 1960's, and were found to be largely limited to those patients with certain health care exposures, have however been also recognized among previously healthy members of the community that lack health care exposures (Herold *et al.,* 1998). Therefore, community acquired MRSA (CA-MRSA) emerged throughout the world in the late 1990's (Otto, 2007).

Research has shown that *S. aureus* has for the past five decades acquired resistances to previously effective's antibiotics that includes penicillinase-resistant ones like methicillin (Diekema *et al*., 2001). The case has however becomes worsen, that, in the present day, MRSA now poses a serious therapeutic problem in the entire world (Engemann*etal*.,2003). MRSA are strains of *S aureus* that shows altered penicillin binding protein (PBP2a) causing conferring resistances to beta-lactam antibiotics (Arunava *et al*., 2013). MRSA are stubborn and notorious, because of their wide variations in antibiotic resistances patterns. They have consistently develop chromosomal resistances to penicillins and cephalosporins, as well as have frequently shown resistances to the wide range of antibiotics commonly prescribed in the hospitals (Pavillard *et al*.,1982).The resistance pattern of prevalent MRSA strains are tantamount to continuous changes over a period of time, as a result of the changes in antibiotic prescription patterns, as well as other factors like control measures and awareness among health care workers (Arunava *et al*., 2013). Antibiotics pressure continue to increase in various hospitals, this invariably leads the emergence of new strains with higher antibiotics resistance replacing the previous strains.

MRSA might be a serious worldwide pathogen, studies are largely restricted to affluent regions of the world, giving rise to very limited information regarding the frequency and characteristics of MRSA in developing countries.

II. MATERIALS AND METHODS

Sampling : Two hundred and ninety-two (292) swab samples was collected from clinically diagnosed chronic skin ulcer patients between January 2012 to January, 2013 from four hospitals in Kaduna state, which include, Ahmadu Bello University Teaching Hospital, Zaria, Barau Dikko Specialist Hospital, Kaduna, General Hospital, Kafanchan and Hajiya Gambo Sawaba Genaral Hospital, Zaria. The swab samples were collected from Surgical Out- patients' Department (SOPD) clinics, General Out-patients' Department (GOPD) clinics, Orthopedics and Dental clinics across the four hospitals. Cases were recruited consecutively from the hospitals.

Ethical Approval: Ethical approval was obtained from the authorities of the ABUTH, Zaria and from the Ministry of Health, Kaduna state, to cover the other three hospitals. Informed consent was however obtained from the patients concerned.

Isolation of *Staphylococcus aureus* : The samples were cultured on blood agar and mannitol salt agar (MSA) plates using the streak method. The plates were incubated at 37^{o}C , the blood agar plates were observed after 24 hours, while the mannitol salt agar were observed after 48 hours for to allow for visible growth (Cheesbrough, 2002). Sub- culturing was carried from the agar plates in order to obtain pure cultures of the isolates. Thereafter, distinct well-separated yellow colonies and creamy white colonies on MSA and blood agar respectively were picked aseptically and stored on agar slants, which were used for further characterization.

Biochemical characterization of *Staphylococcus aureus* isolates : The isolates were subjected to some preliminary tests, such as, Gram stain, Catalase and Coagulase tests, before further biotyping to specie level using the Microgen Staph ID Kit (UK), following the manufacturer's instructions.

Antibiotic Susceptibility Testing: Before the Antibiotic Susceptibility testing was carried out, the isolates were sub-cultured as in the case of the biochemical tests by sub-culturing onto fresh Nutrient agar slants incubated at 37^{0}C for 24 hours. Suspensions were prepared from the sub-cultured isolates into clean, sterilized tubes according to 0.5 McFarland's standard using *S. aureus* ATCC 25923 as positive control strain. The isolates were then tested for their susceptibility to 10 different antibiotics: Co-trimoxazole (25µg), Gentamicin (10µg), Cefoxitin (30µg), Tetracycline (30µg), Ciprofloxacin (5µg), Chloramphenicol (30µg), Erythromycin (15µg), Vancomycin (30 µg) and Amoxicillin-clavulanic acid (30 µg). The antibiotic discs (Liofilchem, Italy) were gently pressed to make sure they are in contact with the inoculated Mueller - Hinton agar surface, and the plates were incubated at 37°C for 18-24 hours. The zones of inhibition were measured after incubation to the nearest millimeter. The interpretation of the zones of inhibition was done using the chart adapted from Clinical and Laboratory Standards Institute (CLSI), 2007.

III. RESULTS

The total number of *Staphylococcus aureus* isolated from the chronic skin ulcer patients from the four hospitals sampled was eighteen (18), of which 14(78%) was Methicillin resistant *Staphylococcus aureus* (MRSA), as shown in the figure below.

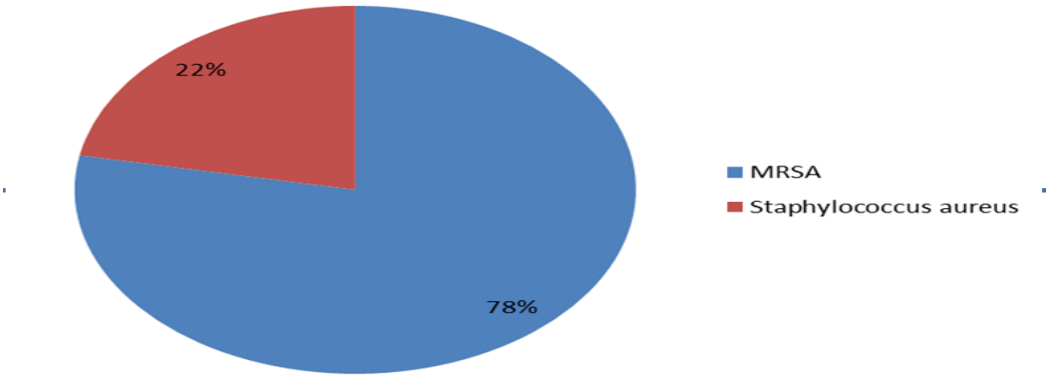

Fig1: Distribution of *Staphylococcus aureus* isolated from chronic skin ulcer.

Table 1: Distribution of MRSA isolates on the sites of infection.

Site of Infection	No of Isolates
Leg	08
Hand	02
Mouth region	02
Abdominal region	02
Ear	00
Breast	00
Buttocks	00

Table 1 is a reflection of the MRSA isolates on the different sites of infection. The part of the infected skin with the highest no of isolates is the leg (lower extremity ulcer).There was no isolate found on Ear, Breast and Buttocks.

Table 2: Antibiotic Resistance Pattern of MRSA to the Antibiotics

MRSA	No of antibiotics resisted	Antibiotics Resistance Pattern
Isolate 2	3	FOX, AUG, SXT
3	3	FOX, AUG, C
5	2	FOX, AUG
6	4	FOX, AUG, TE, SXT
7	4	FOX, AUG, TE, SXT
10	3	FOX, AUG, SXT
11	2	FOX,
12	4	FOX, AUG, SXT, TE
13	6	FOX, AUG, E, TE, SXT, C
14	8	FOX, AUG, VA, CN, E, TE,CIP,SXT
15	6	FOX, AUG, VA, E, CIP, SXT
16	6	FOX, AUG, VA, TE, SXT, C
17	4	FOX, AUG, VA, SXT
18	4	FOX, AUG, SXT, TE

Key: MRSA = Methicillin resistant *Staphylococcus aureus*

Table 2 shows the MRSA isolates that exhibits resistance to other antibiotics as much as they resisted cefoxitin. Common among these antibiotics are Amoxicillin-clavulanic acid (Augmentin), Trimethoprim-sulphamethoxazole and Tetracycline. The commonest antibiotic resistant pattern exhibited by MRSA is in the order of Cefoxitin (FOX) Augmentin (AUG), Tetracycline (TET) and Trimethoprim-sulphamethoxazole (SXT).

TABLE 3:Percentage Resistance of MRSA to the Antibiotics.

ANTIBIOTICS	MRSA n= 14
Cefoxitin(30µg)	77.8
Amoxicillin - Clavulanic acid(30µg)	100.0
Vancomycin(30µg)	28.6
Gentamicin(10µg)	7.0
Erythromycin(15µg)	21.4
Tetracycline(30µg)	57.4
Ciprofloxacin(5µg)	14.3
Trimethoprim – Sulfamethoxazole(25µg)	78.6
Chloramphenicol(30µg)	21.4

n = no of isolates tested

Table 3 shows the resistance of Methicillin resistant *Staphylococcus aureus* (MRSA) isolates to the antibiotics. MRSA isolates in this study exhibited highest resistance to Amoxicillin-Clavulanic acid (Augmentin), in addition to the resistance displayed towards cefoxitin. The isolates however showed the least percentage resistance to gentamicin, meaning they are highly susceptible to the antibiotic.

IV. DISCUSSION

Wound contaminants mostly bacteria are capable of causing deterioration in the wounds, and possibly causing delay in wound healing. These contaminants might be from the environment or introduced through traumatic injury (Bowler, 2001). *Staphylococcus aureus* isolated from the chronic skin ulcer cases in this study amounts to eighteen (18) isolates, out of which methicillin resistant *Staphylococcus aureus* (MRSA) totaled 14 (78%). The presence of *Staphylococcus aureus* in the chronic skin ulcer as reported in this study might not be unconnected with its presence in the environment, and subsequently introduced into the chronic wound through skin abrasion or injury. This result conformed to the work of Gjadsbal *et al.,* (2006) which affirmed that *S. aureus* is frequently isolated from chronic leg ulcer. In a similar study, Gjadsbal *et al.,* (2013) reported that *S. aureus* was found in 13 out of 16 chronic skin ulcer cases. Negar *et al.,* (2012), affirmed that *S. aureus* is a major cause of hospital acquired infection worldwide. MRSA has been reported to a major public health problem worldwide (Jarvis *et al.,* 2007). The fact that MRSA predominate the *Staphylococcus aureus* isolated in this study is an indication of possible indiscriminate use of drug or drug abuse by the patients. Most patients also don't complete their dose, possibly assuming that they have recovered from their injuries. The burden of MRSA has been on the increase, Nimimo *et al.,* 2006, reported a growth rate of 14% of all *S. aureus* strains from clinically significant samples in New South Wales, Australia. The emergence of MRSA in the hospitals, particularly in this study is not surprising, this is because there is the probability that the MRSA so isolated from the patients are from the community where these patients live. In other words, MRSA could find its way to the hospital environment via community-hospital cross-infection and vice versa. There is the probability that the health care workers may carry MRSA on their hands, and/or clothes, since they are always in contact with the asymptomatic carriers or patients who have clinical infection. Through this means, the unsuspecting health workers may transmit MRSA to other patients. Apart from this, the source of MRSA infection could equally be from contaminated environmental surfaces.

The different areas of the body affected by the chronic skin ulcer in the present study where swab samples were taken include the leg (lower extremity) ulcer, hand, abdominal region and the mouth (buccal cavity). The leg however appeared to be the site mostly colonized with MRSA, and the commonest part of the body with chronic skin ulcer. The reason for this could be due to the fact that the leg is arguably one of the most active part of human body, as it is involved in various activities such as sports, recreational activities, driving etc. Owing to this, the leg is often exposed to dangers and therefore prone to injury that could likely be infected.

Various reports from bacterial flora of leg ulcers in patients admitted to the hospital in a research study carried out by Maria *et al.,* (2005), indicated that 80% to 100% of chronic ulcers are at some point colonized by *Staphylococcus. aureus*, making the bacteria the most predominant microorganism isolated from leg ulcer swabs.

Cefoxitin antibiotic which is currently used as a surrogate antibiotic to methicillin was found to be resisted by most of the *S. aureus* isolates, to a percentage of 78. It is worthy to note that, it is on the basis of *S. aureus* resistance to this antibiotic that *S. aureus* is been classified as MRSA and MSSA. Resistance to cefoxitin could influence resistance to other antibiotics by the *S. aureus* isolate, now MRSA, since they have shown resistance to cefoxitin. This could be the reason why the MRSA isolates showed resistance to other antibiotics in this study, such as, Amoxicillin-clavulanic acid, Trimethoprim-sulphamethoxazole and Tetracycline. The antibiotic that was majorly resisted by MRSA is Amoxicillin-clavulanic acid (Augmentin), the MRSA isolates showed 100% resistance to Augmentin. Martins *et al.,* (2012), reported the 100% resistance of *S. aureus* isolates from venous leg ulcers to cefoxitin, to support the findings in this study. As revealed in this study, tetracycline was resisted by the MRSA isolates by more than 50%, this agrees with the result of Abdullah *et al.,* (2007), that revealed the resistance of MRSA isolate to tetracycline by as much as 79%. In another development, MRSA isolates was also reported to be 60% resistant to tetracycline and Trimethoprim-sulphamethoxazole. In a similar vein, Ruhe *et al.,* (2007), also reported the resistance of MRSA isolates to Trimethoprim-sulphamethoxazole by as much as 67%. In a little deviation from this, Al-Anazi and Awadh (2009), working on the prevalence of MRSA in a hospital setting, found out that the isolates were more than 50% resistance to Trimethoprim-sulphamethoxazole. Resistance pattern of MRSA isolates to anti-staphylococcal drugs in past and recent years indicated that the isolates were resistant to tetracycline and trimethoprim-sulphamethoxazole by 50% and 77.5% respectively (Arunava *et al.,* 2013).

In conclusion, MRSA are found to be gradually developing resistance to most antibiotics, hence an alternative treatment of MRSA would indeed be necessary to treat chronic skin ulcers. It has therefore become imperative to develop new antibiotics to combat the isolates. The source of MRSA in this research study might not be unconnected with the fact that the study area is an hospital setting, which leads to nosocomial infection (HA-MRSA), and eventually infections in the community (CA-MRSA).

Acknowledgement

I would like to thank the medical personnel in the various hospitals where the samples were taken, most especially the Nurses who assisted in the area of sample collection from the patient as dictated by the guidelines of the Ethical approval.

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