

Full Length Research Paper

Reoccurrence and distribution of methicillin-resistant *Staphylococcus aureus* (MRSA) in clinical specimens in Bauchi, North eastern Nigeria

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Abstract

Nosocomial infection caused by methicillin resistant staphylococcus aureus (MRSA) presents with management difficulties in infected patients due to their resistance to a number of other frontline antibiotics and constitutes significant epidemiological problems. This study was undertaken to determine the prevalence of methicillin resistant S. aureus and antibiotic sensitivity pattern in clinical isolates in Bauchi. There is dearth of information on this subject in Bauchi. One hundred and fifty (150) S. aureus isolates from various clinical specimens obtained over a 12-month period in the Microbiology Department of Specialist Hospital Bauchi, Bauchi state were subjected to methicillin susceptibility testing, while including susceptibility testing to other antibiotics by the disc diffusion method. Out of 150 S. aureus isolates tested, 42(28.0%) were found to be methicillin resistant. While 24(57.1%) MRSA isolates were obtained from in-patients, 18(42.0%) MRSA were from out-patients. Female patients had 27 (64.3%) MRSA isolates, while male patients had 15 (35.7%) MRSA isolates. Urine sample had the highest prevalence of 17 (40.5%) MRSA isolates, while the least was from ear swab with 4 (2.7%), and there was no MRSA isolated from nasal swab. Antibiotics sensitivity results of methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) with common antibiotics Gentamicin and Ciprofloxacin were encouraging. A prevalence of 28.0% MRSA in this environment calls for urgent intervention strategies due to its possible rapid spread and therapeutic problem.

Keywords: MRSA, Prevalence, Antibiotic susceptibility, Bauchi.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of *S. aureus* that is resistant to methicillin or to virtually all available beta-lactam antimicrobials and other antibiotics i.e. multidrug resistant, very difficult to treat and susceptible only to glycopeptides antibiotics such as vancomycin and teicoplanin (Mehta et al., 1998) or with some new and expensive drugs like linezolid, tigecycline, daptomycin that all

have limitations (Andreoletti et al., 2009). This resistance is mediated by the *mecA* gene, chromosomally located in the staphylococcal cassette chromosome (SCC_{mec}), which codes for a penicillin binding protein (PBP)2a with a low affinity for beta-lactams (Andreoletti et al., 2009; IWG-SCC, 2009). The pathogenicity of *S. aureus* infections is associated with various bacterial surface components (e.g., capsular polysaccharide and protein A), including those recognizing adhesive matrix molecules (e.g., clumping factor and fibronectin binding protein), and to extracellular proteins (e.g., coagulase, hemolysins, enterotoxins, toxic-shock

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syndrome (TSS) toxin, exfoliatins, and Panton-Valentine leukocidin (PVL) (Archer, 1998) and MRSA strains being group of *S. aureus* are likely to have one or more of these pathogenicity traits.

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first isolated just one year after the introduction of methicillin into clinical use in 1960 (Barber, 1961; Jevons, 1961), and by the 1990s increased dramatically worldwide, becoming a serious clinical problem in hospital environments. In recent years a major change in epidemiology of MRSA has been observed, with the appearance of cases in the community affecting people having no epidemiological connection with hospitals (Andreoletti et al., 2009). Prolonged hospital stay, indiscriminate use of antibiotics, lack of awareness, receipt of antibiotics before coming to hospital etc are the possible predisposing factors of MRSA emergence (Anupurba et al., 2003). However, there has been an explosion in the number of MRSA infections reported for populations lacking risk factors for exposure to the health care system (Adam et al., 2007; Baggett et al., 2003; (Buckingham et al., 2004). This increase has been associated with the recognition of new MRSA strains, often called community-associated MRSA (CA-MRSA) strains, which have been responsible for a large proportion of the increased disease burden observed in the last decade. These CA-MRSA strains appear to have rapidly disseminated among the general population in most areas of the United States and affect patients with and without exposure to the health care environment (David and Daum 2010).

Community-associated MRSA strains have been distinguished from their health care-associated MRSA (HA-MRSA) counterparts by molecular means. HA-MRSA strains carry a relatively large staphylococcal chromosomal cassette *mec* (SCC*mec*) belonging to type I, II, or III. These cassettes all contain the signature *mecA* gene, which is nearly universal among MRSA isolates. They are often resistant to many classes of non- β -lactam antimicrobials. HA-MRSA strains seldom carry the genes for the Panton-Valentine leukocidin (PVL). In contrast, CA-MRSA isolates carry smaller SCC*mec* elements, most commonly SCC*mec* type IV or type V. These smaller elements also carry the *mecA* gene and are presumably more mobile, although few explicit data support this notion (Berglund and Söderquist 2008). They are resistant to fewer non- β -lactam classes of antimicrobials and frequently carry PVL genes (David and Daum 2010).

MRSA can be transmitted between people and animals during close contact (Loeffler and Lloyd 2010; Weese et al., 2006) and has been recognized as a zoonotic disease in many countries (Battisti et al., 2010; Schwarz et al., 2008; Bagcigil et al., 2007; Wulf et al., 2008). MRSA isolates are genetically

heterogeneous (Fitzgerald et al., 2001) Some strains, which are called epidemic strains, are more prevalent and tend to spread within or between hospitals and countries (Lee 2003). Other "sporadic" strains are isolated less frequently and do not usually spread widely. Some clonal lineages of *S. aureus* have a tendency to colonize specific species, and may be adapted to either humans or animals (21). Other lineages, which are called "extended host spectrum genotypes," are less host-specific and can infect a wide variety of species (Cuny, et al., 2010). For example, the isolate MRSA ST22-IV (EMRSA15) has been reported in people, dogs, cats, bats, turtles, pigs (rarely) and birds (Cuny, et al., 2010; (van den Broek et al., 2009).

The increasing prevalence of MRSA multi-drug resistant strains which limits the therapeutic options available for the management of MRSA associated infections has become a worrisome issue worldwide (Terry-Alliet et al., 2011). Results from studies at different locations in Nigeria viz: Zaria (Onanuga et al., 2005), Oshogbo (Olowe et al., 2007), Kano (Nwankwo et al., 2010) revealed higher prevalence rates of 69.0%, 47.8% and 28.6% respectively. Since variation in prevalence and resistance exist across different environmental conditions, we therefore determined the prevalence of MRSA from different clinical specimens and their *in vitro* susceptibility pattern to various antimicrobial agents to record their status of MRSA response to commonly used antibiotics in the region.

MATERIALS AND METHODS

This study was carried out in the Specialist Hospital, Bauchi, Northeastern Nigeria.

Sample Collection

Organisms from clinical samples were cultured as per the routine procedures. A total of 150 consecutive non-repeat clinically significant *Staphylococcus aureus* from 68 urine, 23 high vaginal swabs, 19 wound swabs, 12 urethral swabs, 10 seminal fluid, 7 sputum, 5 endo-cervical swabs, 4 ear swabs, and 2 nasal swabs sampled for analysis.

Bacteriology

Each sample was inoculated (in duplicates) into mannitol salt agar plates and incubated at 37°C for 24 h. The characteristic isolates were aseptically isolated, characterised, and identified as *Staphylococcus aureus* by established microbiolo-

Table 1. Distribution of *S. Aureus* and *MRSA* isolates by source

Source	<i>S. Aureus</i> No. (%)	<i>MRSA</i> no. (%)
Urine	68 (45.3)	17 (40.5)
High vaginal swab	23 (15.3)	7 (16.7)
Wound swab	19 (12.7)	5 (11.9)
Urethral swab	12 (8.0)	5 (11.9)
Seminal fluid	10 (6.7)	4 (9.4)
Sputum	7 (4.7)	1 (2.4)
Endo-cervical swab	5 (3.3)	2 (4.8)
Ear swab	4 (2.7)	1 (2.4)
Nasal swab	2 (1.3)	0 (0.0)

Table 2. Age distribution of patients with *MRSA* positive isolates

Age group (Years)	<i>MRSA</i> (%)
1-2	0 (0.0)
11-20	4 (9.5)
21-40	15 (35.7)
41-60	23 (54.8)
>60	0 (0.0)

gical procedures and conventional biochemical tests including: colony morphology (size and pigment), Gram staining, catalase test, coagulase tests, and manitol fermenting (Cheesbrough B 2006).

Antimicrobial susceptibility testing

The antimicrobial susceptibility pattern of the *S. aureus* isolates was determined using Kirby-Bauer-NCCLS modified disc diffusion technique (Cheesbrough, 2006). All the confirmed *S. aureus* strains were subsequently tested for methicillin resistance based on Kirby-Bauer disk diffusion method using oxacillin discs (1µg) obtained from Oxoid. The isolates were considered methicillin resistant if the zone of inhibition was 10 mm or less. Furthermore, the antibiotic susceptibility pattern of methicillin-resistant *S. aureus* strains was determined on the day of their isolation by the modified Kirby Bauer disc diffusion method on Muller Hinton agar using the criteria of standard zone sizes of inhibition to define sensitivity or resistance to different antimicrobials. The antibiotics used were gentamycin (10µg), ciprofloxacin (10µg), sparfloxacin (5µg), ofloxacin (10µg), erythromycin (10µg), azithromycin (15µg), chloramphenicol (30µg), and streptomycin (30µg). The diameter of the zone of inhibition produced by each antibiotic disc was measured, recorded and the isolates were classified as "resistant", "intermediate" and "sensitive" based on

the standard interpretative chart updated according to the current NCCLS standard (BSAC, 2002; NCCLS, 2002).

RESULTS

Out of 150 isolates of *Staphylococci* obtained from different clinical specimens from 150 patients out of which 42 (28%) were methicillin-resistant. Table 1 shows the prevalence distribution of various clinical specimens of samples with methicillin-resistance *Staphylococcus aureus* (*MRSA*) and Coagulase positive *Staphylococcus aureus* (*COSA*). Highest prevalence of 68 (45.3%) SA and 17 (40.5%) *MRSA* was isolated from urine, while nasal swab with 2 (1.3%) SA and no *MRSA* had the least prevalence distribution. Table 2 shows the age distribution of patients with *MRSA*. The age group of 41 – 60 had the highest 23 (54.8%) out of 42 *MRSA* isolated, followed by 21 - 40 and 11 – 20 age groups with 15 (35.7%) and 4 (9.5%) respectively. Table 3 shows the antibiotic resistance pattern of Methicillin-resistant strains of *Staphylococcus aureus*. Highest susceptibility of 38 (90.5) was observed against Gentamycin, followed by Ciprofloxacin 37 (88.1%), Sparfloxacin 36 (85.7), Ofloxacin 33 (78.6), Erythromycin 21 (50.0%). Least susceptibility (highest resistance) was observed against Streptomycin 29(69.0%), Chloramphenicol 25 (59.5%), and Azithromycin 24 (57.1). Table 4 shows the distribution of *MRSA* isolates by admission and

Table 3. Antibiotic sensitivity pattern of MRSA isolates from clinical specimens (n=42)

Antibiotics tested	Sensitive	MRSA isolated (%)
		Resistant
Gentamicin (10µg)	38 (90.5)	4 (9.5)
Ciprofloxacin (10µg)	37 (88.1)	5 (11.9)
Sparfloxacin (5µg)	36 (85.7)	6 (14.3)
Oflaxacin (10µg)	33 (78.6)	9 (31.4)
Erythromycin (10µg)	21 (50.0)	21 (50.0)
Azithromycin (18µg)	18 (42.9)	24 (57.1)
Chloramphenicol (30µg)	17 (40.5)	25 (59.5)
Streptomycin (30µg)	13 (31.0)	29 (69.0)

Table 4. Distribution of MRSA isolates by admission status and sex

	Male No. (%)	Female No. (%)
Out Patients	5 (11.9)	13 (31.0)
In Patients	10 (23.8)	14 (33.3)
Total	15 (35.7)	27 (64.3)

sex. Of the 42 MRSA isolates, 24(57.1%) were obtained from inpatients while outpatients had 18 (42.9%). Female patients had 27 (64.3%) while male patients had 15(35.7%).

DISCUSSION

Methicillin resistance detection in staphylococci can be problematic in the clinical microbiology laboratory because of the heterogeneity of the bacterium under test. The detection of resistance in these isolates has been troubled due to variability in standard techniques used in determining methicillin resistance (Terry-Alliet al., 2011). Although it was not possible to carry out a polymerase chain reaction (PCR) to detect *mecA* gene which is regarded as the gold standard for determining methicillin resistance, sensitivity to or not with 5µg methicillin discs (Oxoid) were used. This was the same method adopted by the other studies used for comparison with this present report. In this study, a prevalence rate of 28.0% MRSA was recorded. This is lower than the study in Zaria (Onanuga et al., 2005), Oshogbo (Olowe et al., 2007), and Kano (Nwankwo et al., 2010), which revealed higher prevalence rates of 69.0%, 47.8%, and 28.6% respectively.

Human-adapted, hospital strains of MRSA are rare among people in the Netherlands and Scandinavian countries, where extensive control programmes are in effect (Kluytmans, 2010; Leonard et al., 2008). Danish control programs decreased the

percentage of MRSA among *S. aureus* from 15% in 1971 to 0.2% in 1984 (Leonard et al., 2008). In the Netherlands, less than 1% of *S. aureus* isolates from clinical specimens are methicillin resistant, and nasal carriage occurs in 0.03% of people admitted to the hospital (excluding people with risk factors for zoonotic carriage) (Leonard et al., 2008). In contrast, more than 50% of human *S.aureus* isolates were reported to be methicillin resistant in Korea in the early 2000s (Lee, 2003). In the U.S., approximately 1.5% of the population carried MRSA in 2003-2004 (Otter, et al., 2010). One recent U.S. study reported that, overall, 5.6% of its study population was colonized (Kottler, et al., 2010). From the foregoing, it is clear the MRSA has become a global nosocomial pathogen with attendant therapeutic problems.

Since complete eradication of MRSA may not be possible, control of transmission seems to be the appropriate goal. The efficacy of some controlling methods are widely recognized and recommended by most authors. The first and the most effective way among these are to avoid transmission through hand contamination from personnel even to patients. The use of broad-spectrum antibiotics for treating infections also increases the rate of MRSA and other resistant bacteria. Therefore chemotherapy should be guided by sensitivity of the probable causative organism. Accurate detection of MRSA by clinical laboratories is of great importance; also awareness should be created about the route of its transmission in the community and the risk factors for infection such as antimicrobial and parental drug use.

CONCLUSION

In conclusion, the degree of resistance or sensitivity of MRSA towards commonly used antibiotics is recognized to be diverse from region to region. The use of antibiotics inevitably requires the need for *in vitro* susceptibility testing of every isolate of MRSA in the clinical laboratories. Our study is a preamble to enable epidemiologists to understand the nature of MRSA isolates in this part of Nigeria. There should be an effective infection control committee to coordinate implementation of its policies especially regular hand washing and strict ward antisepsis to reduce nosocomial infections. Although vancomycin resistant MRSA is not yet common in this part of the world, the rate of spread of this pathogen and its unique ability to acquire and transfer antibiotic resistance calls for urgent and well coordinated surveillance programme to combat this situation. Therefore, there should also be strict antibiotic prescription policies enforced by the appropriate authorities to contain the abuse of antibiotics and reduce acquisition of resistance by pathogens. Educational awareness should be encouraged to update health care workers with new intervention strategies.

ACKNOWLEDGEMENT

We are grateful to all staff in the Microbiology unit of the Specialist Hospital Bauchi, Bauchi state.

REFERENCES

- Adam H, McGeer A, Simor A (2007). Fatal case of post-influenza community-associated MRSA pneumonia in an Ontario teenager with subsequent familial transmission. *Can. Commun. Dis. Rep.* 33: 45 – 48
- Anupurba S, Sen MR, Nath G, Sharma BM, Gulati AK, Mohapatra TM (2003). Prevalence of methicillin-resistant *Staphylococcus aureus* in a Tertiary care Referral Hospital in Eastern Uttar Pradesh. *Indian J Med Microbiol* 21: 49–51.
- Archer G (1998). *Staphylococcus aureus*: a well-armed pathogen. *Clinical Infectious Disease* 26:1179-1181.
- Bagcigil FA, Moodley A, Baptiste KE, Jensen VF, Guardabassi L (2007). Occurrence, species distribution, antimicrobial resistance and clonality of methicillin and erythromycin-resistant *Staphylococci* in the nasal cavity of domestic animals. *Vet. Microbiol.* 121(3-4): 307 – 315
- Baggett HC, Hennessy TW, Leman R, Hamlin C, Bruden D, Reasonover A, Martinez P, Butler AC (2003). An outbreak of community-onset methicillin-resistant *Staphylococcus aureus* skin infections in Southwestern Alaska. *Infect. Control Hosp. Epidemiol.* 24: 397 – 402
- Barber M (1961). Methicillin-resistant *Staphylococci*. *J. Clin. Pathol.* 14: 385-93.
- Battisti A, Franco A, Merialdi G, Hasman H, Jurescia M, Lorenzetti R, Feltrin F, Zini M, Aarestrup FM (2010). Heterogeneity among MRSA from Italian pig finishing holdings. *Vet. Microbiol.* 1142(3-4):361 – 366
- Berglund C, Söderquist R (2008). The origin of a MRSA isolate at a neonatal ward in Sweden: Possible horizontal transfer of a Staphylococcal cassette chromosome *mec* between MRS *Haemolyticus* and *Staphylococcus aureus*. *Clin. Microbiol. Infect.* 14: 1048 – 1056
- Buckingham SC, McDougal LK, Cathey LD, Comeaux K, Craig AS, Fridkin SK, Tenover FC (2004). Emergence of community-acquired MRSA at a Memphis, Tennessee Children's hospital. *Pediatr. Infect. Dis.* 23: 619 – 624
- Cuny C, Friedrich A, Kozytyska S, Layer F, Nübel U, Ohlsen K, Strommenger B, Walther B, Wieler L, Witte W (2010). Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in different animal species. *Int. J. Med.* 300(2-3):109 – 117
- David MZ, Daum RS (2010). Community-Associated MRSA: Epidemiology and clinical consequences of an emerging epidemic. *Microbiol. Rev.* 23(3): 616 – 687
Doi:10.1128/CMR.00081-09
- Fitzgerald JR, Sturdevant DE, Mackie SM, Gill SR, Musser JM (2001). Evolutionary genomics of *Staphylococcus aureus*: Insights into the origin of methicillin-resistant strains and the toxic shock syndrome epidemic. *Proc. Natl. Acad. Sci. USA.* 95(15): 8821 – 8826
- International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC) (2009). Classification of Staphylococcal Cassette Chromosome *mec* (SCC*mec*): Guidelines for Reporting Novel SCC*mec* Elements-IWG-SCC, *Antimicrob. Agents Chemother.* 53: 4961-4967.
- Jevons M (1961). Celbenin resistant *Staphylococci*. *BMJ* 1: 124-125.
- Kluytmans JA (2010). Methicillin-resistant *Staphylococcus aureus* in food products: cause for concern or cause for complacency? *Clin Microbiol Infect.* 16(1):11-5.
- Kottler S, Middleton JR, Perry J, Weese JS, Cohn LA (2010). Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* carriage in three populations. *J. Vet. Intern. Med.* 24(1):132- 9.
- Lee JH (2003). Methicillin (Oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. *Appl. Environ. Microbiol.* 69(11): 6489 – 6494
- Lee JH (2003). Methicillin (Oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. *Appl. Environ. Microbiol.* 69(11): 6489-94.
- Leonard FC, Markey BK (2008). Methicillin-resistant *Staphylococcus aureus* in animals: a review. *Vet J.* 175(1):27-36.
- Loeffler A, Lloyd DH (2010). Companion animals: a reservoir for methicillin-resistant *Staphylococcus aureus* in the community? *Epidemiol. Infect.* 138(5):595 – 605
- Mehta AP, Rodrigues C, Sheth K, Jani S, Hakimiyan A, Fazalbhoy N (1998). Control of methicillin resistant *Staphylococcus aureus* in a tertiary care Centre: A five-year study. *J Med Microbiol.* 16:31–4
- Andreoletti O, Budka H, Buncic S, Colin P, Collins JD, DeKoeijer A, Griffin J, Havelaar A, Hope J, Klein G, Kruse H, Magnino S, López M, McLauchlin J, Nguyen-The C, Noeckler K, Noerrung B, Maradona MP, Roberts T, Vågsholm I, Vanopdenbosch E (2009). Assessment of the Public Health significance of methicillin-resistant *Staphylococcus aureus* (MRSA) in animals and foods: Scientific Opinion of the Panel on Biological Hazards. *European Food Safety Authority Journal.* 993: 1- 73
- National Committee for Clinical Laboratory Standards (2002): Performance standards for antimicrobial disc susceptibility tests. Twelfth International Supplement; M100-S12.
- Nwankwo EOK, Abdulhadi S, Magagi A, Ihesiulor G (2010). Methicillin resistant *Staphylococcus aureus* (MRSA) and their antibiotic sensitivity pattern in Kano, Nigeria. *Afr. J. Clin. Exper. Microbiol.* 11(1):129-136
- Cheesbrough B (2006). District

- Laboratory practice in Tropical Countries Part 2, Second Edition
Cambridge University Press, Cambridge
- Olowe OA, Eniola KIT, Olowe RA, Olayemi AB (2007). Antimicrobial susceptibility and Beta- lactamase detection of MRSA in Osogbo, South western Nigeria. *Nature and Science* 5(3):44 – 48 ISSN:1545-0740
- Onanuga A, Oyi AR, Onaolapo JA (2005). Prevalence and susceptibility pattern of methicillin-resistant *Staphylococcus aureus* isolates among healthy women in Zaria, Nigeria. *Afri. J. Biotech.* 4(11): 1321 – 1324
- Otter JA, French GL (2010). Molecular epidemiology of community-associated methicillin-resistant *Staphylococcus aureus* in Europe. *Lancet Infect. Dis.* 10(4):227-39.
- Schwarz S, Kadlec K, Strommenger B (2008). MRSA and *Staphylococcus pseudintermedius* detected in the BfT-GermVet monitoring programme 2004 – 2006 in Germany. *J. Antimicrob. Chemother.* 61(1): 282 – 285
- Terry-Alli OA, Ogbolu DO, Akorede E, Onemu OM, Okanlawon BM (2011). Distribution of *mecA* gene amongst *Staphylococcus aureus* isolates from South-western Nigeria. *Afri. J. Biomed. Res.* 14(1):9 – 16
- van den Broek IV, van Cleef BA, Haenen, A., Broens, E.M., van der Wolf, P.J., Huijsdens, X.W., van den Broek, MJ, van de Giessen AW, Tiemersma EW (2009). Methicillin-resistant *Staphylococcus aureus* in people living and working in pig farms. *Epidemiol. Infect.* 137(5):700 -70
- Weese JS, Dick H, Willey BM, McGeer A, Kreiswirth BN, Innis B, Low DE (2006). Suspected transmission of MRSA between domestic pets and humans in veterinary clinics and in the household. *Vet. Microbiol.* 115(1-3):148 – 155
- Wulf MW, Tiemersma E, Kluytmans J, Bogaers D, Leenders AC, Jansen MW, Berkhout J, Ruijters E, Haverkate D, Isken M, Voss A (2008). MRSA carriage in healthcare personnel in contact with farm animals. *Hosp. Infect.* 70(2): 186 – 190