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Surveillance of Methicillin Resistant *Staphylococci* in Patients and their Hospital Care Providing Relatives in Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria

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ABSTRACT

Background

Staphylococcal infections remain a problem the world over. Two important staphylococcal virulence and drug resistance factors are PVL and PbP2a. Constant surveillance for these factors can improve treatment outcomes and reduce cost and duration of hospitalization.

Objectives

The aim of this study was to carry out surveillance of methicillin resistant *Staphylococci* among patients and their hospital care-giving contacts, and to provide an update on susceptibility pattern of the local isolates to anti-staphylococcal antibiotics.

Methods

Skin and nasal specimens were collected from patients and their hospital caregivers and processed by standard microbiological and molecular techniques.

Results

Recovery rate of MRSA was 23% while MRCoNS was 5% (t = 9.1875; P = 0.00004661). Nine (18%) of *S. aureus* isolates were SpA^- strains, while 1 (2%) was OS-MRSA. Of 35 MRSA isolates, 16 (53%) possessed PbP2a protein. Three of the $PbP2a^+$ MRSA and 4 of MSSA isolates were VISA strains. Thirteen (43%) of isolates tested had Oxacillin MIC $\geq$256µg/ml, while all (40 or 100%) were susceptible to Teicoplanin (MIC range = 0.06-4µg/ml). Of 25 *S. aureus* isolates tested for PVL gene, 6 harbored the gene; majority (67%) of the pv gene – bearing *S. aureus* were of MSSA phenotypes.

Conclusion

Teicoplanin appears to be the drug of choice for empirical treatment of methicillin-resistant *Staphylococcal* infections in our locality. A report of SpA^- *S. aureus* and OS-MRSA isolates are being made for the first time in this region and raises the need to include PBP2a detection among routine laboratory tests on Staphylococcal isolates.

Keywords: Methicillin-Resistant *Staphylococci*, PbP2a, PVL gene, Nigeria

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INTRODUCTION

Staphylococcus aureus is a normal inhabitant of the human body and colonizes the nasopharynx of many healthy humans [1]. The organism can cause a wide array of human diseases, ranging from relatively benign skin infections to life-threatening debilitating conditions affecting different anatomic sites of the body, including outbreaks [2], [3]. Of the *Staphylococci*, coagulase-positive species (referred to as *S. aureus)* is the major cause of human infections.

There is considerable genetic and phenotype diversity among natural populations of *S. aureus*, with remarkable strain-dependent variations in production of molecules involved in host-pathogen interaction [4]. Among such molecules are supplemental Penicillin Binding Protein (known as PBP2a) and Panton Valentine Leukocidin (PVL), which are products of Mec and Luk genes, respectively. Panton Valentine Leukocidin is considered an important factor in staphylococcal virulence, while PBP2a promotes resistance to therapeutic agents.

With the introduction of benzyl penicillin into clinical use in 1940s, β-lactam antibiotics became the mainstay therapy against staphylococcal infections; this is due to the potent activity and good safety profile of the drug against *Staphylococci* [5]. Beta-lactam antibiotics act by inhibiting the synthesis of peptidoglycan – a molecule that is crucial for structural integrity of bacterial cell wall. Synthesis of cell well peptidoglycan is mediated by membrane-bound enzymes, collectively known as penicillin-binding proteins (PBPs) – most of which are transpeptidating enzymes [5]. The mechanism of action of β-lactam antibiotics is through irreversible acylation of the serine residue of the active site of the catalytic PBPs; the loss of catalytic activity of PBP leads to formation of bacterial cell walls with reduced functional integrity, which is lethal to the bacterium [6]. However, shortly after the introduction of penicillin into clinical use, resistant strains of *S. aureus* began to emerge against the drug, due to production of penicillin-inactivating enzyme, referred to as penicillinase (or β-lactamase). This problem was initially overcome by introduction of a penicillinase-stable penicillin known as methicillin, but in 1961 methicillin resistance also appeared among isolates of *S. aureus* [7]. Since then, methicillin-resistant *S. aureus* (MRSA) and, lately, methicillin-resistant coagulase-negative *Staphylococci* (MRCoNS) have become recognized as important problems in hospitals and intensive care units, worldwide [8].

The persistent problem of methicillin-resistance among *Staphylococci* has necessitated recourse to alternative anti-staphylococcal agents, such as glycopeptides (e.g. Vancomycin and Teicoplanin), lipopeptides (e.g. Daptomycin), and oxazolidinones (e.g. Linezolid). But susceptibility of organisms to antimicrobial agents can vary from one locality to another (and can also change over time in a particular locality) as can etiologic factors. This calls for continual updates and surveillance for factors that can affect disease and treatment outcomes.

The aim of this study, therefore, was to carry out surveillance of methicillin resistant *Staphylococci* among patients and their hospital care providing contacts in Nnamdi Azikiwe University Teaching Hospital, Nnewi Nigeria; this is with the objectives of investigating the distribution of methicillin resistant *Staphylococci* in the hospital wards, determining PBP2a and Luk-PV gene status of isolates, and providing an update on the susceptibility of local isolates to standard anti-staphylococcal therapeutic agents.

METHODS

Subjects, samples collection, and sample processing

This was a cross-sectional study, in which patients admitted to the medical and surgical wards of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Nigeria, and their hospital care-providing relatives were screened for presence of Methicillin-resistant *Staphylococci* on the body, between 2017 and 2018.

To be considered for inclusion into the study, the subject: was a patient on admission at NAUTH or a care-providing relative of an admitted patient; was not having any on-going antimicrobial treatment, had spent seven or more days in the hospital ward, and gave informed consent to the study. A total of 76 patients and 56 care-providing relatives of patients met these inclusion criteria and were recruited into the study. Ethical approval was obtained from NAUTH ethics committee. Questionnaires were administered to the subjects; information sought through the questionnaires included gender, length of stay in the hospital, history of antimicrobial drugs, among others. Samples were collected from skin (of the hand) and nasopharynx, using sterile cotton wool swabs moistened with sterile ringer's solution. All samples were promptly transported to Professor Emele's research

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laboratory, Faculty of Medicine, Nnamdi Azikiwe University, Nnewi, Nigeria, for analysis.

In the laboratory, the swabs were inoculated onto Mannitol salt agar (MSA), Oxacillin resistance screening agar (ORSA), and into Mueller-Hinton's broth – supplemented with 6.5% NaCl (MHB). Over-night growths on the enrichment medium (MHB) was subsequently sub-cultured onto MSA and ORSA media and examined for growth after 24 hrs incubation at 35^0C. Suspected colonies on the media were identified as *S. aureus* or Coagulase-negative *Staphylococcus* by Gram staining, coagulase test, and other standard identification criteria [9], [10]. Staphaurex latex agglutination test kit (Remel, UK) was used to confirm presence of Protein A. Presence of PBP2a protein was determined, using PbP2a latex agglutination kit (Remel, UK).

Molecular detection of Panton Valentine Leucocidin (PVL) gene

Luk-pv oligonucleotide primer pair used was as follows - based on previously published sequences [10]:

5ˈ- ATCATTAGGTAAAATGTCTGGACATGATCCA-3ˈ F

3ˈ- GCATCAAGTGTATTGGATAGCAAAAGC-5ˈ R.

The primers were obtained from Inqaba Biotec Laboratory, Nigeria.

Genomic DNA extraction, PCR protocols, and PCR thermal profiles were based on standard techniques [11] and according to reagents manufacturers' specifications. The amplified products were analyzed by agarose gel (2% w/v) electrophoresis and ethidium bromide staining; one hundred base pairs (100bp) molecular weight DNA standard (ladder) was used to determine size of the PCR amplicon. The expected product size was 433bp.

Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) of Oxacillin, Linezolid, Vancomycin, Teicoplanin, and Daptomycin were determined, using MIC evaluator gradient diffusion strips (concentration range = 0.015 - 256 µg/ml) (Oxoid, England); susceptibility test procedures and interpretative criteria were by standard methods [12], [13].

Statistical analysis

Data were analyzed, using the statistical package for social sciences (SPSS: 21.0). Confidence interval was set at 95% (α risk of 0.05).

RESULTS

Results showed that 15 (19.7%) of hospital patients, and 15 (26.8%) of patients' hospital care – providing relatives harbored MRSA on the body (X^2 = 1.0322; P = 0.2502). On the contrary, MRCoNS was more commonly isolated from patients (6 or 7.9%) than hospital care providing relatives (1 or 1.8%), although the difference was not statistically significant (X^2 = 1.3664; P = 0.2424), as shown in Table1. Thirty of fifty (60%) of *S. aureus* encountered were MRSA; overall, MRSA was encountered significantly more than MRCoNS from the subjects (t = 9.1961; P = 0.00004661) – (Table 1). Of the MRSA isolates, 16 (53%) produced PbP2a (Table 1). There was no significant difference between patients and their hospital care-giving relatives in the carriage of MRSA (X^2 = 1.0322; P = 0.2502), MRCoNS (X^2 = 1.3664; P = 0.2424) or $PbP2a^+$ MRSA phenotype (X^2 = 0.6189; P = 0.4315).

Table 1: Distribution of different phenotypes of *Staphylococcus* among patients and their hospital care providing relatives in Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Nigeria.

Source of Sample	**No. of Subjects**	**Number (% isolation rate) of Staphylococcus phenotype**				
		MRSA			**MSSA**	**MRCoNS**
		$PbP2a^+$	**$PbP2a^-$**	**Total**		
A. Status of subject						
Hospital Patients	76	9 (11.8)	6 (7.9)	15 (19.7)	8 (10.5)	6 (7.9)

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Patients' caregivers	56	7 (12.5)	8 (14.3)	15 (26.8)	12 (21.4)	1 (1.8)
Total	**132**	**16 (12.1)**	**14 (10.6)**	**30 (22.7)**	**20 (15.2)**	**7 (5.3)**
B. Gender of subject						
Male	61	9 (14.8)	5 (8.2)	14 (23.0)	6 (9.8)	3 (4.9)
Female	71	7 (9.9)	9 (12.7)	16 (22.5)	14 (19.7)	4 (5.6)
Total	**132**	**16 (12.1)**	**14 (10.6)**	**30 (22.7)**	**20 (15.2)**	**7 (5.3)**
C. Hospital Ward						
Male Surgical Ward	40	7 (17.5)	3 (7.5)	10 (25.0)	6 (15.0)	3 (7.5)
Female Surgical Ward	27	1 (3.7)	5 (18.5)	6 (22.2)	7 (25.9)	2 (7.4)
Male Medical Ward	34	6 (17.6)	2 (5.9)	8 (23.5)	3 (8.8)	0 (0.0)
Female Medical Ward	31	2 (6.5)	4 (12.9)	6 (19.4)	4 (12.9)	2 (6.5)
Total	**132**	**16 (12.1)**	**14 (10.6)**	**30 (22.7)**	**20 (15.2)**	**7 (5.3)**

MRSA= Methicillin Resistant *Staphylococcus aureus*; MSSA= Methicillin Susceptible *S. aureus*

MRCoNS = Methicillin Resistant Coagulase Negative *Staphylococci*;

$PbP2a^+$ = Penicillin binding protein–positive phenotype; $PbP2a^-$ = Penicillin binding protein – negative phenotype

Isolates of $PbP2a^+$ MRSA were more often recovered in patients located in male wards (18%) than those in female wards (5%) – $X^2 = 4.6894$; $P = 0.03035$ (Table 2). Recovery rate of methicillin resistant Staphylococci was slightly higher in surgical wards (31%) than in medical wards (25%), although the difference was not statistically significant ($X^2 = 0.74054$; $P = 0.3895$), as shown in Table 2.

Table 2: Rate of isolation of Methicillin Resistant *Staphylococci* in Patients and their Hospital Care providing Relatives in NAUTH, Nnewi, Nigeria

Ward Participant		**No. (%) of Phenotypes recovered**			
		MRSA			**MRCoNS**
		$PbP2a^+$	**$PbP2a^-$**	**Total**	
MSW	Patient (n =26)	3 (11.5)	2 (7.7)	5 (19.2)	2 (7.7)
	Care giver (n = 14)	4 (28.6)	1(7.1)	5 (35.7)	1 (7.1)
FSW	Patient (n = 15)	0 (0.0)	2 (13.3)	2 (13.3)	2 (13.3)
	Care giver (n = 12)	1(8.3)	3 (25.0)	4 (33.3)	0 (0.0)
MMW	Patient (n =20)	5 (25.0)	1(5.0)	6 (30.0)	0 (0.0)
	Care giver (n = 14)	1 (7.1)	1 (7.1)	2 (14.3)	0 (0.0)
FMW	Patient (n =15)	1 (6.7)	1 (6.7)	2 (13.3)	2 (13.3)
	Care giver (n = 16)	1 (6.3)	3 (18.8)	4 (25.0)	0 (0.0)
Total (n = 132)		**16 (12.1)**	**14 (10.6)**	**30(22.7)**	**7 (5.3)**

$PbP2a^+$ = PbP2a positive; $PbP2a^-$ = PbP2a negative; MRSA = Methicillin Resistant *S. aureus*;

MRCoNS = Methicillin Resistant Coagulase Negative *Staphylococci*

MSW = Male Surgical Ward; FSW = Female Surgical Ward;

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MMW = Male Medical Ward; FMW = Female Medical Ward

Of 25 *S. aureus* isolates tested for PVL gene, 6 (24%) harbored pvl gene, 4 (67%) of which were of MSSA phenotypes (Tables 3).

Table 3: Distribution of Luk-pv gene among 25 randomly selected *Staphylococcus aureus* isolates from patients and their caregivers in NAUTH, Nnewi, Nigeria.

***S. aureus* Oxacillin Phenotype Susceptibility isolated profile**		**No. isolated from:**		**Recovery rate of Luk- PV gene**		
		Pt	**Cg**	**Pt**	**Cg**	**Total**
PbP2a$^+$ (MRSA)	Resistant	6	9	1/6 (16.7)	1/9 (11.1)	2/15 (13.3)
	Susceptible	1	0	0/1 (0.0)	0/0 (0.0)	0 /1 (0.0)
	Total	**7**	**9**	**1/7 (14.3)**	**1/9 (11.1)**	**2/16 (12.5)**
PbP2a$^-$	Resistant (MRSA)	2	3	1/2 (50.0)	0/3 (0.0)	1/5 (20.0)
	Susceptible (MSSA)	0	4	0/0 (0.0)	3/4 (75.0)	3/4 (75.0)
Grand Total		**9**	**16**	**2/9 (22.2)**	**4/16 (25.0)**	**6/25 (24.0)**

PbP2a$^+$: PbP2a-positive; PbP2a$^-$: PbP2a-negative; MRSA: Methicillin Resistant *Staphylococcus aureus*;

MSSA: Methicillin Susceptible *S. aureus;* PVL: Panton Valentine Leukocidin

Pt: Patient; Cg: Caregiver

Table 4 highlights the susceptibility pattern of isolates to antimicrobial drugs. Of 12 PbP2a$^+$ *S. aureus* phenotype tested, one was Oxacillin susceptible (OS-MRSA), while the rest were resistant (MIC_{range} = 4 – >256µg/ml; MIC_{90} = >256µg/ml). Fourteen of the PbP2a$^-$ MRSA were Oxacillin-resistant (MIC_{range}= 4 – >256µg/ml; MIC_{90} = >256µg/ml). Oxacillin MIC for MRCoNS ranged from 64 – >256µg/ml (MIC_{90}>256µg/ml). In general, MIC_{90} for Vancomycin, Linezolid, and Daptomycin were slightly higher for PbP2a$^+$ phenotype of MRSA than for PbP2a$^-$phenotype (Table 4). Seven isolates (3 of PbP2a$^+$ MRSA, and 4 MSSA) had Vancomycin intermediate susceptibility (VISA) (Table 4).

Table 4: Minimal Inhibitory Concentration of Antimicrobial drugs against phenotypes of *Staphylococci* isolated from patients and care providing relatives in NAUTH, Nnewi, Nigeria.

***Staphylococcus* Phenotype**	**Minimal Inhibitory concentration (MIC) of antimicrobial agents (µg/ml)**					
	MIC category	**Antimicrobial agent**				
		Oxacillin	**Vancomycin**	**Linezolid**	**Daptomycin**	**Teicoplanin**
PbP2a$^+$ MRSA (n =12)	Range	1 – >256	<0.015 – 16	<0.015 – 8	1 – 16	0.25 – 2
	MIC_{50}	32	1	2	2	1
	MIC_{90}	>256	4	4	16	2
PbP2a$^-$ MRSA (n=14)	Range	4 – >256	<0.015 – 2	0.25 – 4	0.125 – 8	0.5 – 2
	MIC_{50}	64	0.06	2	1	1
	MIC_{90}	>256	2	2	8	2
MSSA (n=10)	Range	0.06 – 2	0.015 – 4	2 – 8	0.125 –16	0.06 – 4
	MIC_{50}	1	4	2	1	1
	MIC_{90}	2	4	8	16	2

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MRCoNS (n = 4)	Range	64 – >256	0.03 – 2.0	1.0 – 2.0	0.5 – 4	0.03 – 2
	MIC_{50}	128	0.06	1	2	0.25
	MIC_{90}	>256	2	2	4	2

$PbP2a^+$: PbP2a-popsitive; $PbP2a^-$: PbP2a-negative; MRSA: Methicillin Resistant *S. aureus*; MRCoNS: Methicillin Resistant Coagulase Negative *Staphylococci*; MIC_{50}: Minimal inhibitory concentration for 50% inhibition; MIC_{90}: Minimal inhibitory concentration for 90% inhibition

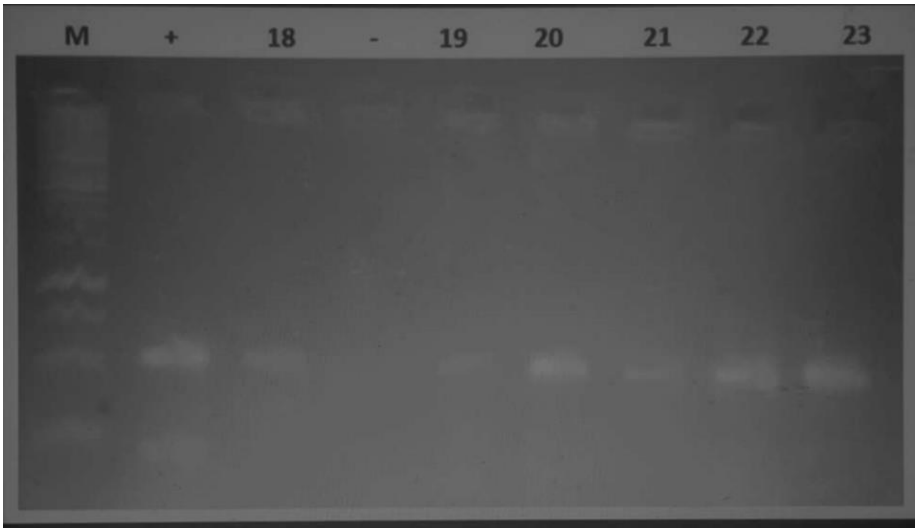

Fig. 1: Picture showing gel electrophoresis of PCR-amplified Luk-pv gene (433bp) of *S. aureus*:

"M" – 100bp DNA ladder

"+" – Positive control (with band at 433bp)

" – " – Negative control (without band)

Lanes 18, 19, 20, 21, 22, and 23 – Positive for pvl (with bands at 433bp)

DISCUSSION

Nnamdi Azikiwe University Teaching Hospital is a tertiary health care institution, with over 500 beds, and serves as a major referral center for people dwelling in 4 states of Nigeria, with estimated population of over 20 million. In this part of the world, when a patient is admitted to the hospital, family members often go along with him/her to provide care for the basic needs. We deemed it necessary to investigate hospitalized patients, along with their care providing family members, for presence of methicillin resistant Staphylococci on the body.

We did not notice any statistically significant difference in carriage of methicillin resistant Staphylococci by patients and their hospital care providing relatives ($X^2 = 0.0142$; $P = 0.9051$), possibly because both groups were equally exposed to the hospital environment. However, results of this study tended to suggest that hospital care providing contacts of patients can help in perpetuating staphylococcal infection in the hospitalized patients – possibly providing some negative feedbacks to any therapeutic efforts in the ward.

It was found out that of 50 *S. aureus* isolates recovered in clinical settings, 30 (60%) were methicillin resistant. The predominance of methicillin resistant strains among *S. aureus* isolates from clinical settings is of serious concern; this is because penicillin antibiotics are very valuable, not only because of the safety profile of this class of drugs but also because of its relatively low cost and ready availability in resource limited countries, such as Nigeria. The preponderance of methicillin resistant strains, as noted in this study, would make penicillin class of drugs to be of limited value in the management of patients in this locality, most of which are of poor economic situation and need inexpensive quality drugs, such as the penicillin. Results also tend to suggest that a patient can acquire methicillin resistant Staphylococcal infection from his (or her) own body microbiota or from that of

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a caregiver. We could not, based on the present study protocol, determine whether the carried strains were acquired during the stay in the hospital or were taken from home to the hospital.

We encountered 9 (18%) coagulase-producing Staphylococcal isolates that lacked protein A; other investigators [14] have similarly reported isolation of *S. aureus* lacking protein A (SpA^- strain) in other parts of the world. Absence of protein A in *S. aureus* could be due to absence of spa gene, or to a failure of spa gene expression in the particular *S. aureus* strain – leading to inability to produce the gene product (protein A). Protein A is very important to *S. aureus* because it helps the organism to evade the host immune response [15]; lack of this protein could make *S. aureus* more susceptible to the host's immune response, with consequent abortive infection.

We encountered seven Oxacillin resistant *Staphylococci* that were coagulase negative and we considered them as Oxacillin (methicillin) resistant coagulase negative *Staphylococci* (MRCoNS). Methicillin resistant coagulase negative *Staphylococci* appear to be of increasing clinical significance the world over [8], as supported by the present findings. Coagulase negative *Staphylococci* are commonly considered to be harmless members of the human body flora but have been associated with severe human infections in parts of the world [8], especially if they are drug resistant.

The majority (53%) of our MRSA isolates were $PbP2a^+$ strain (possessed PbP2a) – suggesting that elaboration of this protein is an important mechanism of penicillin resistance by hospital isolates of *S. aureus* in our locality. This figure is higher than the 45% reported earlier by O'Malley *et* al. [16] in Nigeria; abuse of antimicrobial drugs may have contributed greatly to this trend. Antimicrobial drugs are readily abused in therapy in this part of the world, due to high cost of medical treatment in hospitals, in the presence of increasing poverty of the population. The high cost of hospital bills encourages self-medication and quackery, which often leads to indiscriminate therapeutic use of antimicrobials. Such indiscriminate use of antimicrobial drugs could mount selective pressures that favor emergence of resistant strains, more so as PbP2a is known to be readily induced by administration of β-lactam antibiotics [17]. It should be pointed out that production of PbP2a protein by some of our *S. aureus* isolates suggests that those isolates possessed expressible Mec A gene. However, fourteen (46.7%) of the MRSA isolates were resistant to oxacillin, but did not possess PbP2a protein; their resistance to penicillin could probably be due to mechanisms other than elaboration of PbP2a – possibly by elaboration of PbP2c, which is rarely reported in humans [18], and was not sought or by hyper-production of beta-lactamases [19]. Although we did not encounter MRCoNS that produced PbP2a, there are reports of PbP2a-producing MRCoNS in parts of the world [20]. The possible reason for not recording PbP2a-producing MRCoNS could be because we could not induce it. It has been noted that MRCoNS typically produces lower amounts of PbP2a protein and, therefore, requires induction (by exposure to any of the penicillinase-resistant penicillins) in order to produce sufficient product to be detected by the PbP2a test kit used [21], [22]. Our results also showed that all isolates of MRCoNS were susceptible to Vancomycin, which agrees with the view [8] that Vancomycin is the standard therapy for infections caused by MRCoNS.

We encountered one *S. aureus* isolate (from a patient) that was phenotypically MSSA but produced PbP2a protein; the isolate was safely considered as MRSA (ie. Oxacillin susceptible MRSA or OS-MRSA), since the PbP2a would confer on it the potential to resist methicillin and other antibiotics [19]. Reports on OS-MRSA have been made in other parts of the world [23], [24], and we are reporting it for the first time in this locality. Our OS-MRSA isolate showed intermediate vancomycin susceptibility (VISA) but was susceptible to teicoplanin, daptomycin, and linezolid.

Three of the $PbP2a^-$ MRSA isolates were in the Oxacillin MIC range of 4-16µg/ml. These could be borderline oxacillin resistant *S. aureus* (BORSA), or Modified *S. aureus* (MOD-SA). Resistance in BORSA is due to hyper-production of β-lactamases; on the contrary, resistance in MODSA is associated with alterations (point mutation) in the existing normal PBPs, with consequent reduction in affinity for β-lactam antibiotics [25]; these modifications typically involve PBP3 and/or PBP4, and result from the selective pressure of beta-lactam antibiotics [19].

One of the care-givers on whose body $PbP2a^+$ *S. aureus* was recovered was a female staff nurse. Clonal analysis on isolates would have identified possible lines of transmission within the wards, or relatedness between patients' strains and those of their caregivers; however, clonal analysis was outside the scope of this study. The recovery of $PbP2a^+$ *S. aureus* from the care-givers seem to represent potential hazard to patients in the ward – because

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dissemination from such an individual to hospitalized patients could occur and would complicate treatment of the affected patient. We did not notice any gender-related pattern in the carriage of methicillin resistant Staphylococci in this survey ($X^2 = 0.0015$; $P = 0.9691$).

We also noticed that luk-pv gene occurred both in strains involved in infectious process, as well as those involved in carriage state. However, it should be pointed out that presence of luk-pv gene does not necessarily suggest production of Panton Valentine Leucocidin (which is the product of luk-pv gene); a gene may be present in a cell but may not be expressed, and therefore may not elicit the gene product.

Resistance pattern of the isolates was relatively high for daptomycin, compared with other front-line anti-MRSA drugs. This apparently high level of Daptomycin resistance may partly be as a result of inherent error in the method of antimicrobial susceptibility testing (AST) adopted by us (ie. gradient diffusion strips or E test); previous authors [26] noted that E-test method of AST may give erroneous MIC with Daptomycin, compared with broth microdilution method. However, we did not design for the use of broth microdilution technique in this study.

Many (over 40%) of our isolates of MRSA and MRCoNS showed Oxacillin MIC values higher than 256 µg/ml, which agrees with report from other researchers, using gradient diffusion strips [27], as well as broth microdilution method [28].

We encountered seven Vancomycin intermediate *S. aureus* (VISA) strains. The first *S. aureus* isolate with reduced susceptibility to vancomycin (VISA) was reported in Japan in 1997 [29] and since that report, vancomycin MIC of *S. aureus* has been shown to be on the increase [27], [28], [30]. This "vancomycin susceptibility shift" was reported to be more notable in MSSA than MRSA [30], as is apparent in our result, and may have been facilitated by the common use of vancomycin as front-line anti-MRSA drug. The regular use of vancomycin in therapy could mount selective pressure that could readily convert heterogenous populations (hVISA) to VISA, and subsequently to Vancomycin resistant strains (VRSA). Therefore, judicious use of vancomycin is recommended in order to slow down the apparent "vancomycin MIC surge".

All MRSA and MRCoNS tested were susceptible to teicoplanin, highlighting the promise of teicoplanin in the treatment of drug-resistant Staphylococci in this locality, especially as teicoplanin was also active (at MIC of $\leq$ 2µg/ml) against the seven VISA strains encountered in this study. This result tends to suggest that teicoplanin would be the right choice of drug for empirical treatment in medical emergencies involving Staphylococcal infection in our locality – pending outcome of antimicrobial drug susceptibility test.

CONCLUSION

Based on our results, it could be concluded that patients' hospital care-giving relatives could represent potential danger – not just to the patient they care for in the hospital, but also to other patients in the hospital ward, to the hospital environment, and ultimately to members of their own households (when they return home from the hospital). We therefore recommend regular surveillance for MRSA among the hospital care providers and prompt eradication of any identified carriage state. Presence of OS-MRSA strain in this locality raises the need to include PBP2a detection among routine laboratory tests on Staphylococcal isolates (especially MSSA).

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Conflict of Interest: The authors declare no conflicts of interests.

REFERENCES

1. Piewngam P and Otto M. Staphylococcus aureus colonization and strategies for decolonization. The Lancet Microbe, 2024; 5(6): e606 - e618
2. Emele FE**,** Izomoh MI, and Alufokhai E. Microorganisms associated with wound infection in Ekpoma, Nigeria. West Afr. J. Med.1999; 18(2): 97-100.
3. Wu X, Wang C, He L, Xu H, Jing C, Chen Y et al. Antimicrobial resistance profile of methicillin-

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resistant Staphylococcus aureus isolates in children reported from the ISPED surveillance of bacterial resistance, 2016–2021. Front. Cell. Infect. Microbiol. 2023; 13:1102779. doi: 10.3389/fcimb.2023.1102779

4. Foster TJ. Immune evasion by staphylococci. Nat Rev Microbiol.2005; 3:948–58.
5. Llarrull LI, Fisher JF and Mobashery S. Molecular Basis and Phenotype of Methicillin Resistance in Staphylococcus aureus and Insights into New Beta-Lactams That Meet the Challenge. Antimicrob. Agents chemother. 2009; 53(10): 4051–4063.
6. Hackbarth CJ and Chambers HF. Methicillin-resistant staphylococci: Genetics and mechanisms of resistance. Antimicrob Agents Chemother.1989; 33(7): 991-994.
7. Javon MP. Celbenin – resistant Staphylococci. Br. Med. J. 1961; 1(5219): 124–125.
8. Ekrami A, Montazeri EA, Kaydani GA and Shokoohizadeh L. Methicillin Resistant Staphylococci: Prevalence and susceptibility patterns in a burn center in Ahvaz from 2013–2014. Iran Microbiol.2015; 7(4): 208–213.
9. Cheesbrough, M. Collection, transport, and examination of clinical specimens. In: Medical Laboratory Mannual for tropical countries. Volume II: Microbiology. ELBS edition. Butterworth, Green & Co. (publishers) Ltd. 1985:100 -195.
10. Karmakar A, Jana D, Dutta K and Dua P. Prevalence of Panton-Valentine Leukocidin Gene among Community Acquired Staphylococcus aureus: A Real-Time PCR Study. Hindawi J Path. 2018, Article ID 4518541, 8 pages https://doi.org/10.1155/2018/4518541
11. Darboe S, Dobreniecki S, Jarju S, Jallow M, Mohammed NI, Wathuo M, et al. Prevalence of Panton-Valentine leucocidin (PVL) and antimicrobial resistance in community-acquired clinical Staphylococcus aureus in an urban Gambian hospital: a 11- year period retrospective pilot study. Front cell Infect Microbiol. 2019; 9: https://doi.org/10.3389/fcimb.2019.00170.
12. Mushtaq S, Warner M, Cloke J, Afzal-Shah M, Livermore DM. Performance of the Oxoid M.I.C. Evaluator™ Strips compared with the Etest® assay and BSAC agar dilution. J. Antimicrob Chemother.2019; 65(8):1702–1711, https://doi.org/10.1093/jac/dkq206.
13. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty- Second Informational Supplement. M100-S22. Vol. 32, No. 3; Wayne PA. 2012.
14. Brignoli T, Manetti AGO, Rosini R, Haag AF. Scarlato V, Bagnoli F et al. Delany I. Absence of Protein A Expression Is Associated With Higher Capsule Production in Staphylococcal Isolates. Front Microbiol. 2019; 10, Article 863 www.frontiersin.org
15. Chen X, Shi M, Tong X, Kim HK, Wang L, Schneewind O et al. Missiakas D. Glycosylation-dependent opsonophagocytic activity of staphylococcal protein A antibodies. PNAS. 2020; 117(37):22992–23000.
16. O'Malley, S.M.; Emele, F.E.; Nwaokorie, F.O.; Idika, N.; Umeizudike, A.K.; EmekaNwabunnia, I et al. Molecular typing of antibiotic resistant Staphylococcus aureus in Nigeria. J Infect Publ Health. 2015; 8:187-193.
17. Lade H and Kim JS. Molecular Determinants of β-Lactam Resistance in Methicillin-Resistant Staphylococcus aureus (MRSA): An Updated Review. Antibiotics (Basel). 2023 24;12(9):1362. doi: 10.3390/antibiotics12091362. PMID: 37760659; PMCID: PMC10525618.
18. Larsen J, Raisen CL, Ba X, Sadgrove NL, Padilla-Gonzalez GF, Simmonds, MSJ. et al. Emergence of methicillin resistance predates the clinical use of antibiotics. Nature, 2022 602:135–141. https://doi.org/10.1038/s41586-021-04265-w
19. Hryniewicz MM and Garbacz K. Borderline oxacillin-resistant Staphylococcus aureus (BORSA) - a more common problem than expected? J Med Microbiol. 2017; 66(10):1367-1373.
20. Rey J, Gil M, de Mendoza JH, García A, Gaitskell-Phillips G, Bastidas-Caldes C et al. Clonality and Persistence of Multi-resistant Methicillin-Resistant Coagulase-Negative Staphylococci Isolated from the Staff of a University Veterinary Hospital. Antibiotics (Basel). 2022 Jun 16;11(6):811. doi: 10.3390/antibiotics11060811. PMID: 35740217; PMCID: PMC9220325.
21. Gerberding JL, Miick C, Liu HH and Chambers HF. Comparison of conventional susceptibility tests with direct detection of penicillin-binding protein 2a in borderline oxacillin-resistant strains of Staphylococcus aureus. Antimicrob Agents Chemother. 1991; 35:2574-2579.
22. Chambers, HF. Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Clin. Microbiol. Rev. 1997; 10:781–791.
23. Ranjbar Omid M, Jamali H, Kafilzadeh F, Borjian A and Arzanlou M. Molecular Epidemiology, Virulence Factors, Antibiotic Resistance and Risk Factors for Nasal Carriage of Staphylococcus aureus

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI | Volume XI Issue XV December 2024 | Special Issue on Public Health

in A Teenage Student Population: High Prevalence of Oxacillin Susceptible MRSA Isolates. Jundishapur J Microbiol. 2021; 14(9): e118240. https://doi.org/10.5812/jjm.118240

24. Liu J, Li TM, Zhong N, Wang X, Jiang J, Zhang WX et al. Current status of oxacillin-susceptible mecA-positive Staphylococcus aureus infection in Shanghai, China: A multicenter study. Journal of Microbiology, Immunology and Infection. 2021; 54(6):1070 – 1077.
25. Jorgensen JH. Mechanisms of methicillin resistance in Staphylococcus aureus and methods for laboratory detection. Infect Control Hosp Epidemiol. 1991; 12(1):14-9.
26. Sader HS, Rhomber PR and Jones RN. Nine-Hospital Study Comparing Broth Microdilution and Etest Method Results for Vancomycin and Daptomycin against Methicillin- Resistant Staphylococcus aureus. Antimicrob Agents Chemother. 2009; 53(7): 3162–3165.
27. Martins MA, dos Santos SLV, Leão LSN, Araújo NP, Bachion MM. Prevalence of resistance phenotypes in Staphylococcus aureus and coagulase-negative isolates of venous ulcers of primary healthcare patients. Revist Socied Brasil Med Trop. 2012; 45(6):717-22.
28. Cheddie P, Seepersaud D and Ramlochan T. Detection of Oxacillin (Methicillin)-resistant Staphylococcus aureus Isolated from a Tertiary-care Hospital, Georgetown, Guyana. Microbiol Res J Int. 2020; 29(6): 1-7.
29. Hiramatsu K, Aritaka N, Hanaki H, Kawasaki S, Hosoda Y, Hori S, et al. Dissemination in Japanese hospitals of strains of Staphylococcus aureus heterogeneously resistant to vancomycin. Lancet, 1997; 350(9092):1670-3.
30. Wang G, Hindle JF, Ward KW and Bruckner DA. Increased Vancomycin MICs for Staphylococcus aureus Clinical Isolates from a University Hospital during a 5-Year Period. J Clin Microbiol. 2006; 44(11): 3883–3886.