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METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) ISOLATES IN ILORIN, NIGERIA

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Nosocomial infections caused by methicillin-resistant strains of *Staphylococcus aureus* often pose therapeutic dilemma to the clinicians because of the multi resistant nature of these strains of *Staphylococcus aureus*. Outbreaks of both nosocomial and community acquired infections are also frequent and difficult to control. This study determined the prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* (MRSA) at the University of Ilorin Teaching Hospital, between January and December 2001. The methicillin disc diffusion method for the detection of methicillin resistance and the Kirby-Bauer disc diffusion for antibiotic susceptibility tests, were used. The MRSA prevalence rate was 34.7% (51/147) of all *Staphylococcus aureus* isolates. Forty-five isolates were associated with infections and 6 were colonizing strains. Thirty-six (70.6%) were hospital (nosocomial) acquired while 15 (29.4%) were community-acquired. Forty-eight patients have received antibiotics previously including 30 who had received multiple antibiotics. Skin and soft tissues were sites of infections in 36 cases and surgical, emergency and intensive care units accounted for 31 isolates. All MRSA isolates were resistant to more than two antibiotics but remained largely susceptible to third generation cephalosporins, macrolides and quinolones and all were sensitive to vancomycin. We recommend the use of third generation cephalosporins and quinolones where indicated, in the treatment of serious MRSA infections in this environment. Control of the spread of MRSA in this hospital must include reinforcement of appropriate use of antibiotics, hand washing and laboratory surveillance for MRSA, particularly in the surgical wards and intensive care units, in order to identify sources of outbreaks.

Keywords: Methicillin-resistant, Staphylococcus aureus, Ilorin.

INTRODUCTION

In recent times, strains of methicillin resistant *Staphylococcus aureus* (MRSA) have become a source of great concern to Clinical Microbiologists because these strains are now established nosocomial pathogens in health care facilities worldwide (1). These strains which appeared in Europe soon after the introduction of methicillin for β-lactam resistant *Staphylococcus aureus* in 1960 (2), and in the United States a decade later (3), were initially associated with sporadic outbreaks in hospitals, and generally sensitive to other antibiotics. However since the 1970s, these strains have been replaced by multiply resistant MRSA, which are now endemic in hospitals and are difficult to eradicate (4).

World wide, MRSA comprise up to 50% of *Staphylococcus aureus* isolates in hospitals (5), and is involved in surgical wound infections, nosocomial pneumonias, bacteraemias and other forms of infections (6-9). Treatment options have been severely restricted to potentially toxic antimicrobials, leading to increase mortality, morbidity and costs (10).

In view of the hetero-resistant nature of most strains of MRSA, routine microbiology laboratories may fail to detect MRSA if sensitivity testing is done at incubation temperatures greater than 35°C and if agar plates are read before 24 hours incubation (4). In spite of this, the overall incidence of MRSA isolation has gradually increased in many developed countries to present levels of about 30% in Spain, France and Italy (11) and up to 54% in Japan (12). In Africa, where routine laboratory testing may particularly fail to detect MRSA strain, it is not surprising that few hospitals report MRSA infection (13-16). In this institution, there is no routine screening for methicillin resistance among clinical isolates of *Staphylococcus aureus*. Previous studies in this centre (17, 18) have implicated *S. aureus* to be predominant Gram positive pathogen involved in nosocomial wound infections, burns, bacteraemia and septicaemia in all age group.

It is therefore appropriate to determine the contribution of MRSA strains to these infections and determine their susceptibility pattern. These would serve as guides to therapeutic approach and in formulation of control policy for MRSA in this environment.

MATERIALS AND METHODS

Patients

Hospitalized patients of all age groups and sexes in different wards and intensive care units with clinical features suggestive of sepsis were recruited into the study. Also outpatients attending the hospital general outpatient department for the first time on account of sepsis or sepsis-related conditions and with no previous hospitalization in the last six months were included in the study.

Specimens/Isolation

Routine clinical specimens made up of sputum, urine, swabs, pus/aspirates, blood and cerebro-spinal fluids were aseptically collected, transported and processed in the microbiology laboratory of the University of Ilorin Teaching Hospital according to standard techniques (19). Colonies of *Staphylococcus aureus* were identified on culture media by a combination of morphology, Gram reaction, positive catalase, tube coagulase and deoxyribonuclease tests.

Methicillin resistance screening

The methicillin disc diffusion test was employed (20). A sample of the test isolates and control strains were grown overnight at 35°C in nutrient broth. The overnight cultures were diluted with sterile saline (0.85% NaCl) in bijou bottles and their turbidity compared to 0.5 McFarland standard using white paper as background. The inocula were then spread with sterile cotton wool swab on Mueller Hinton agar (Oxoid, England) supplemented with 2% NaCl. 5 µg methicillin discs (Oxoid, England) were applied onto the plates with a sterile forcep. The agar plates were incubated for full 24 hours at 35°C aerobically and then inspected for growth. The diameter of zone of inhibition of each isolate was measured using a calibrated ruler and sensitivity or resistance estimated by comparing with the zone diameter interpretive standard (5). Isolates with zone diameter less than 14 mm to methicillin were considered methicillin resistant strains (5).

Susceptibility to other antimicrobials was carried out using Kirby Bauer disc diffusion method (21). The following single-paper discs were used; Chloramphenicol (30 µg), Erythromycin (15 µg), Tetracycline (30 µg),

Cefotaxime (30 µg), Ciprofloxacin (30 µg), Cotrimoxazole (1.25,23.75 µg), Vancomycin (30 µg), and Penicillin (1 mu). Zone diameter for sensitivity (S) of MRSA for each antibiotic was as defined by the National Committee for Clinical Laboratory Standards (5); Gentamicin (>15 mm), Chloramphenicol (>18 mm), Tetracycline (>19 mm), Cotrimoxazole (>16 mm), Vancomycin (>12 mm), Penicillin (>29 mm), Erythromycin (>23 mm), and Cefotaxime (>23 mm).

Patients bio-data

Where MRSA was isolated, full patient bio-data such as age, sex, types of infection, date of admission, length of admission, history of underlying diseases, operations and procedures, movement of patients in the hospital, previous hospitalization, antimicrobial therapy and outcome of infections were obtained from the request forms and case files. Using standard clinical and microbiologic criteria (22), MRSA was ascertained as infecting or colonizing strain. Where there was clinical evidence of infection, the MRSA was considered as infecting strain and where there was no clinical evidence to suggest the MRSA isolated was responsible for infection or when a strain was isolated from a site not involved in infection, the strain was considered a colonizer.

RESULTS

A total of 147 *Staphylococcus aureus* strains were isolated and characterized from clinical specimens during the study period, out of which 51 were methicillin resistant, giving an MRSA prevalence rate of 34.7%. Thirty-six of the 51 MRSA (70.6%) were acquired in the hospital (nosocomial) while 15 (29.5%) were acquired in the community. All 36 nosocomial strains were associated with clinical infections while 6 of the 15 community strains were colonizers. Surgical wards accounted for 24 of 36 (66.7%) nosocomial isolates, followed by ICU (19.4%), medical wards (8.3%) and emergency unit (5.6%). (Tables 1 and 2)

All the nosocomial strains were isolated from patients who had been hospitalized for at least one week for either illness initially unrelated to MRSA infections or had undergone surgery. All the community-acquired strains were isolated from patients attending the hospital general outpatients for the first time and had no previous hospitalization in the last six months before presentation. Skin and soft tissues were the commonest sites of infection, accounting for 80% of all infections in this study and infections followed surgery or trauma. Other sites of infection were the middle ear in 4 patients, bone in 3 patients and urinary tract in 2 patients (Table 3). The colonizing strains were all community acquired and were recovered in the urinary tract in one patient and upper respiratory tract in five patients.

Forty-eight (48) patients representing 94.1% of patients had used antibiotics within one month before MRSA was isolated. Thirty patients (58.8%) had used 3 or more different antibiotic types, 12 patients (23.5%) had used 2 while 2 patients (11.8%) had used 1 antibiotic previously. Only 3 patients claimed not to have used antibiotics at least six months before MRSA was isolated (Table 3). Ampicillin/cloxacillin was the commonest drug used by patients (37/51), followed by tetracycline (30/51), cotrimoxazole (20/51), chloramphenicol (17/51), quinolones (3/51) and others not specified (10/51).

The antibiotic susceptibility pattern of MRSA is as shown in Table 4. All isolates were sensitive to vancomycin (30 μg) and also moderately sensitive to cefotaxime (82.4%), erythromycin (76.5%) and ciprofloxacin (76.5%).

Fifty two point nine percent of the isolates were susceptible to gentamicin but the isolates were generally resistant to cotrimoxazole, chloramphenicol, penicillin, and tetracycline with 52.9%, 94.1% and 100% of isolates respectively resistant to these agents.

TABLE 1: Distribution of Methicillin-Resistant S.aureus isolates at UITH Ilorin

MRSA	Hospital acquired (%)	Community (%)	
Infecting strain	36(100)	9(60)	45(88.2)
Colonizing strain	0	6(40)	6(11.8)
TOTAL	**36(70.6)**	**15(29.4)**	**51**

TABLE 2: Ward Distribution of isolates at UITH Ilorin

Ward/Unit	No of isolates
Orthpaedic (W2)	10
General Surgery (W5)	14
ICU	7
Emergency (A/E, EPU)	2
Medical (W4, W6, W7)	3
GOPD	15
Total	**51**

ICU – Intensive Care Unit, W – Ward, EPU – Emergency Paediatric Unit

A/E – Accident and Emergency, GOPD – General Outpatient Dept.

Table 3: Characteristics of MRSA isolates at UITH Ilorin

1.	**Types/Sites of infection**	**Number (%)**
	i. Post operative skin/soft tissue infection	36(80)
	ii. Otitis media	4(8.9)
	iii. Osteomyelitis	3(6.7)
	iv. Unitary tract infection.	2(4.4)
2.	**Colonizing strain**	
	i. Upper respiratory tract	5(83.3)
	ii. Urinary tract	1(16.7)
3.	**Previous use of antibiotics**	
	i. 1 antibiotic	6(11)
	ii. 2 antibiotics	12(23.8)
	iii. > 3 antibiotics	30(58.8)
	iv. None.	3(5.9)
4.	**Types of antibiotics**	
	i. Ampicilin/Cloxacillin	37
	ii. Tetracycline	30
	iii. Cotrimozale	20
	iv. Chloramphenicol	17
	v. Quinolones	3
	vi. Others (not specified)	10
	vii. > 3 antibiotic types	30

Table 4: Antibiotic susceptibility pattern of MRSA isolates at UITH Ilorin

Antibiotic types	Number sensitive (%)	Number resistant (%)
Penicillin (1 mega unit)	3(5.9)	48(94.1)
Gentamicin (10μg)	27(52.9)	24(47.1)
Chloramphenicol (30μg)	3(5.9)	48(94.1)
Erythromycin (15μg)	39(76.5)	12(23.5)
Tetracycline (30μg)	0(0)	51(100)
Cefotaxime (30μg)	42(82.4)	9(17.6))
Ciprofloxacin (30μg)	39(76.1)	12(3.5)
Cotrimoxazole (1.25/23.75μg)	24(47.1)	27(52.9)
Vancomycin (30μg)	51(100)	0(0)

DISCUSSION

The MRSA prevalence of 34.7% (51/147) of *Staphylococcus aureus* isolates in this hospital is comparable to the prevalence reported by Bello *et al* in Jos (23), Durmaz *et al* in Turkey (24), Voss and Doebbeling in France, Italy and Spain (11), but less than the prevalence reported by Okèsola *et al* in Ibadan (13), Kesah *et al* in Lagos (14), Sow *et al* in Dakar (16), Loureiro *et al* in Rio de Janeiro (25), and Lotsu *et al* in Japan (12). The prevalence is also higher than that reported in two Somalian hospitals among nasal carriers by Nur *et al* (15), in general hospital, Port of Spain among clinical isolates by Swanston (26), in Saudi University hospital by Bukharie and Abdelhadi (27), and in Scandinavian hospitals (28). The occurrence and frequency of MRSA is known to vary geographically from hospital to hospital and over time depending on the level of infection control practices and antibiotic consumption rates (29). In the United States hospitals, prevalence rates reported vary between 10 and 50% (28).

Twenty nine point four percent (15/51) of the MRSA isolates in the study were community acquired. This is comparable to what obtains in some places (4,30), where these strains contribute significantly to the total MRSA problem. Community acquired MRSA infection is common among narcotic drug addicts (30). However, in all the patients with community acquired infection and colonization in this study none had history of narcotic abuse. Thirty-six of the 45 MRSA infecting strains were hospital acquired and 80% of the infection they caused occurred on the skin and soft tissues following surgery, 8.9% in the middle ear, 6.7% in the bone and 4.4% in the urinary tract. This is in conformity with reports elsewhere (25,. 30). *Staphylococcus aureus* is a normal flora of the skin in most people and expectedly can contaminate wounds or any breach in the integuments to set up a focus of infection. Six of the 15 community acquired MRSA strains were harboured by patients in their respiratory and urinary tracts. These strains were isolated in the course of investigations for suspected pulmonary tuberculosis in 5 of the patients and urinary tract infection in 1 patient, but were found not to be involved in clinical disease. Anterior nares, skin and upper respiratory tracts are known common sites of colonization by MRSA (26, 28).

Staphylococcus aureus is usually found in surgical, intensive and emergency care services and it is therefore not surprising that most (91.7%) of the MRSA isolates in this study were located in these service areas where antibiotic usage is greatest. All but 3 of the patients with MRSA infection have been exposed to antibiotics previously, which included ampicillin/cloxacillin, tetracycline, chloramphenicol and cotrimoxazole and 30 (58.8%) have used 3 or more (multiple) different antibiotic types before developing infection.

All the MRSA isolates were resistant to tetracycline and all but 2 were resistant to chloramphenicol and penicillin, 3 most commonly abused drugs in our environment. However, MRSA isolates here remained largely sensitive to the third generation cephalosporins, macrolides and quinolones. This is at variance with what obtains in some places (11, 24, 26). This observation can be explained by the fact that these drugs are expensive and therefore not readily available and affordable by majority of people here, who

are of low socioeconomic class. A previous study in this hospital showed *Staphylococcus aureus* isolated from wound infections to be generally sensitive to these agents (17). It is however interesting to note the relatively high sensitivity of MRSA in this study to gentamicin (52.9% of isolates sensitive), which though at variance with the trend world-wide, may represent a phenotype of MRSA (GS-MRSA) similar to the ones reported recently in French hospitals (31). Southern blot hybridization with *mec A* and *aa6'-aph2'* gene-specific DNA probes would have confirmed the presence of methicillin resistance gene and the lack of the bifunctional 2' aminoglycoside phosporylase-6-aminoglycoside acetytransferase gene (*aac6' – aph2'*) responsible for gentamicin, tobramycin and amikacin cross-resistance in these strains. Unfortunately there is no such facility in this environment.

All the MRSA isolates in this study were sensitive to vancomycin. This is in agreement with reports elsewhere in this country (13, 14), and in other places (11, 23, 31). Vancomycin has remained the drug of choice for treating serious MRSA infections such as endocarditis and septicaemia for over 40 years (10). This glycopeptide is relatively toxic and needs to be administered by intravenous infusion. There are no published reports on vancomycin usage in our environment and hence resistance is not expected. However Japanese and American researchers have isolated MRSA with reduced susceptibility to vancomycin from patients with MRSA endocarditis who were on intravenous vancomycin therapy (33). It is only a matter of time before vancomycin resistant MRSA becomes another significant epidemiologic problem.

The methicillin disk diffusion susceptibility test to detect methicillin resistance used in this study has been standardized (20). In this study, Mueller-Hinton agar was supplemented with 2% NaCl, 5 μg methicillin disc was used, and plates were incubated for full 24 hours at 35^{0}C. Isolates that initially appeared sensitive when plain Mueller-Hinton agar was used became resistant when supplemented with 2% NaC1. The mechanism of methicillin resistance is complex and still unclear (34). The chromosomal *mec A* gene in MRSA is believed to encode an abnormal penicillin binding protein (PBP 2a) with reduced binding affinity to penicillin and related β-lactam compounds. Expression of the resistance gene is increased in the presence of 2-4% NaCl, pH more than 5.2, incubating temperature of 30-35^{0}C and the presence of β-lactam agents in the medium (34). This study has confirmed some of these assertions.

CONCLUSION/RECOMMENDATION

The prevalence of MRSA in this institution is relatively high. It is therefore imperative to establish a guideline for MRSA detection and control in the hospital by; daily monitoring of clinical laboratory for MRSA isolates, a programme of monthly prospective culture surveillance of inpatients believed to be at high risk for acquisition of MRSA, re-identification of previously colonized or infected patients at the time of subsequent readmission into the hospital, screening of hospital personnel when an outbreak is suspected, routine hand washing by hospital personnel and policy regulation antibiotic prescription and usage. With the unavailability of vancomycin in our environment coupled with its toxicity, we recommend the use of

third generation cephalosporins such as ceftazidime, cefotaxime or ceftriaxone as well as the fluoroquinolones where indicated, in the treatment of serious MRSA infections in this locality.

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