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HIGH INCIDENCE OF MULTIDRUG-RESISTANT STRAINS OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM CLINICAL SAMPLES IN BENIN-CITY, NIGERIA

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RUNNING TITLE: MRSA SHOWS HIGH RATE OF MULTIDRUG RESISTANCE FROM CLINICAL ISOLATES

ABSTRACT

Infections of methicillin-resistant *Staphylococcus aureus* (MRSA) are becoming an increasingly concerning clinical problem. The aim of this study was to assess the development of multidrug resistant strains of MRSA from clinical samples and possibilities for reducing resistance. This study included a total of seventy-five (75) isolates comprising fifteen (15) each collected from ear, urine, cervix, blood and wounds. An agar disc diffusion test was used to measure the effects of antimicrobial agents against the bacteria isolates following standardized guidelines. Out of a total of 75 clinical isolates of *S. aureus* collected, 43 (57.3%) were resistant to methicillin with isolates obtained from ear infections showing the highest resistance pattern of 14.7% while the least was from urine sample with incidence of 5.3%. From the 43 isolates that showed resistance to methicillin, 36 (83.7%) were multidrug resistant to various classes of antibiotics tested. MRSA showed an increasing trend of antimicrobial resistance and therefore calls for periodic surveillance of nosocomial infections due to *S. aureus* and other important bacterial pathogens.

Key Words: methicillin-resistant *Staphylococcus aureus*, MRSA, multidrug resistance, MDR

L'INCIDENCE ELEVEE DE SOUCHES MULTIRESTANTES DE STAPHYLOCOCCUS AUREUS RESISTANT A LA METHICILLINE ISOLÉS DES ECHANTILLONS CLINIQUES A BENIN - CITY, NIGERIA.

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TITRE COURANT: MRSA MONTRE UN TAUX ELEVE DE MULTIRESTANCE DE ISOLATS CLINIQUES.

RESUME

Les infections *Staphylococcus aureus* résistant a la methicilline (MRSA) sont de plus en plus devenir une préoccupation clinique. Le but de cette recherche était d'évaluer le développement des souches multirésistantes de MRSA des échantillons clinique et les possibilités de réduire la résistance. Cette recherche a compris un total de soixante quinze (75) isolats comprenant quinze (15), chaque collecté de l'oreille, de l'urine, du col de l'utérus, du sang et des plaies. Un test de diffusion sur disque d'agar a été employé pour mesurer les effets des agents antimicrobiens contre les isolats bactériens selon les directives normalisées. Sur un total de 75 isolats cliniques de *S. aureus* collectés, 43 (57,3%) étaient résistants a methicilline avec des isolats obtenus des infections de l'oreille montrant le profil de résistance le plus élevé de 14,7% tandis que le moins était de l'échantillon d'urine avec une incidence 5,3%. Des 43 isolats qui ont montré la résistance amethicilline, 36(83,7%) étaient multirésistants aux diverses classes d'antibiotiques testés. MRSA a montré une tendance de plus en plus de la résistance aux antimicrobiens et demande par conséquent la surveillance périodique des infections nosocomiales due a *S. aureus* et d'autres pathogènes bactériens importants.

Mots - clés: *Staphylococcus aureus* résistant a la methicilline, MRSA, Multrésistance , MDR.

INTRODUCTION

Among all the antibiotic resistance achieved by *Staphylococcus aureus*, two most remarkable ones are methicillin and vancomycin resistance. The methicillin resistance was achieved by interspecies transfer of *mecA* gene from an ancestral *Staphylococcus* species to *S. aureus* mediated by a unique staphylococcal mobile genetic element. Vancomycin resistance was achieved by horizontal transfer of a plasmid-born *vanA*-gene transposon from vancomycin-resistant Enterococcus to *S. aureus* across the genus barrier. Practically all *S. aureus* isolates were methicillin susceptible until 1961, when Jevons found three MRSA strains among 5440 clinical *S. aureus* strains in England (1).

MRSA is born when methicillin-susceptible *S. aureus* (MSSA) has acquired the methicillin-resistance gene *mecA* by horizontal gene transfer mediated by a mobile genetic element staphylococcal cassette chromosome (SCC) (2). *S. aureus* colonizes various parts of healthy humans such as the nares, skin, vagina and gastrointestinal tract (3). Its prevalence has been severally reported in healthy populations; 36% and 40% were reported in women's urine in two centres in Nigeria, 17.3% in nasal cavity of Turkish children, 36% in nares of Japanese adults and 32.4% in nasal cavity of adults in USA (4-7). Colonizing strains may serve as endogenous reservoirs for overt clinical infections or may spread to other patients.

S. aureus have become resistant to various antimicrobial agents including the commonly used penicillin-related antibiotics. Multi-drug resistant strains of *S. aureus* have been reported with increasing frequency worldwide. Strains that are resistant to methicillin were found to exhibit varying resistance to lincosamides, macrolides, aminoglycosides, fluoroquinolones, or combinations of these antibiotics (8, 9). Vancomycin – a glycopeptide which was initially very effective in the treatment of methicillin resistant *S. aureus* (MRSA) infections is recently being witnessed with intermediate resistance from MRSA strains (10).

In this study we hypothesize that the constant use of antibiotics in the hospitals could result in high amount of multidrug-resistant strains of MRSA

STUDY AREA

Clinical isolates of *Staphylococcus aureus* from patients' samples were obtained from the Medical Microbiology Laboratory of the University of Benin Teaching Hospital, Benin City, Nigeria within a 3-month period from July – September, 2007.

SAMPLE PROCESSING

A total of seventy-five (75) isolates were collected comprising fifteen (15) each from ear, urine, cervix, blood and wounds. Identification and confirmation of isolates were conducted based on growth and fermentation on mannitol salt agar (MSA), colonial morphology, Gram staining and positive results to both catalase and coagulase tests (11).

SUSCEPTIBILITY TEST

The susceptibility of isolates to oxacillin using the E-test strips (AB Biodisk) was carried out by the disk diffusion method (12). Also tested were commercial antibiotics; amoxicillin 30µg, ampicillin/cloxacillin 30µg, ceftriaxone 25µg, cefuroxime 20µg, ciprofloxacin 10µg, pefloxacin 10µg, gentamicin 10µg, streptomycin 30µg, erythromycin 10µg and sulphamethoxazole/trimethoprim 30µg. Methicillin discs 5µg (Oxoid, England) was applied onto the Petri dish alongside with other tested antibiotics. A breakpoint of ≥ 2 µg was used to define resistance to oxacillin, zone diameter less than 14mm for methicillin while multidrug resistance was defined as strains resistant to three or more drug classes other than beta-lactams.

RESULTS

Out of a total of seventy-five (75) clinical isolates of *S. aureus* collected in this study, 43 representing 57.3% were resistant to methicillin. The distribution according to the site of isolation is shown in Table 1. Isolates obtained from ear infections showed the highest resistance pattern of 14.7% while the least was from urine sample with incidence of 5.3%. Figure 1 shows the resistance pattern of the various isolated to the antibiotic classes with multidrug resistance defined as resistance to three or more classes of antibiotics other than the beta-lactams. From the 43 isolates that showed resistance to methicillin, 36 (83.7%) were multidrug resistant.

TABLE 1: PREVALENCE OF MRSA FROM DIFFERENT CLINICAL SAMPLE

Site	No. of <i>S. aureus</i> isolates	No. resistant to methicillin/oxacillin	Percentage(%)
Ear	15	11	14.7
Cervix	15	10	13.3
Urine	15	4	5.3
Blood	15	10	13.3
Wound	15	8	10.7
Total	75	43	57.3

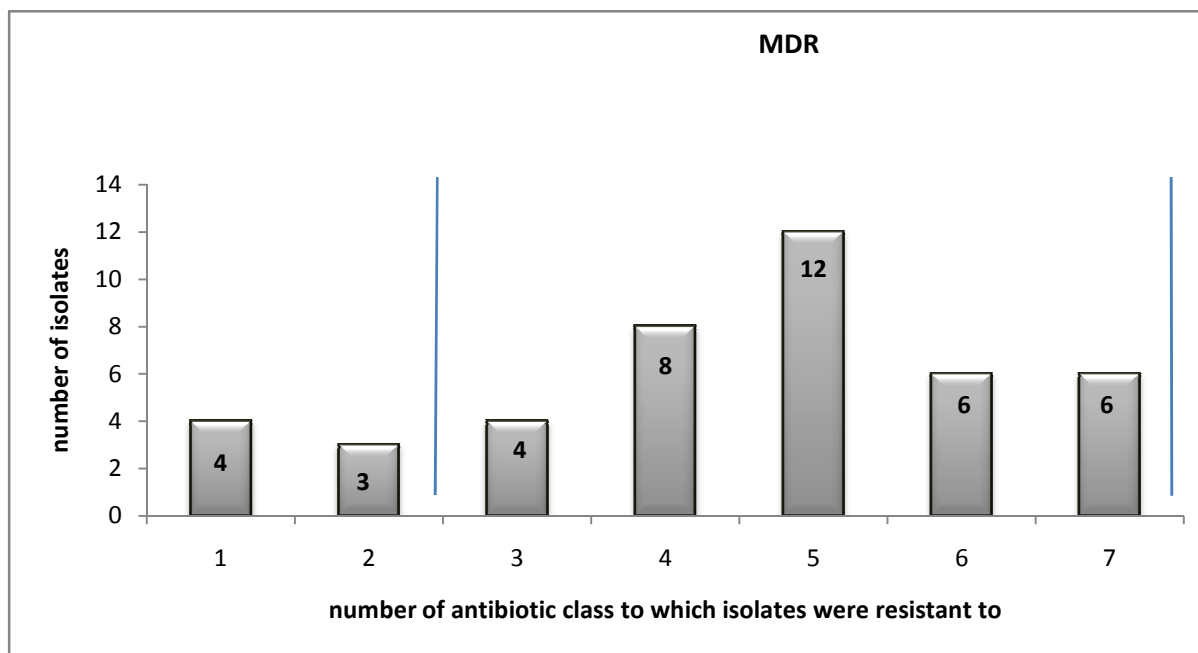


FIGURE 1: RESISTANCE PATTERN OF ISOLATES TO THE VARIOUS ANTIBIOTICS TESTED.
(3-7) - multidrug resistance, N = 36

DISCUSSION

Infections caused by multi-resistant strains of *S. aureus* are identified by their resistance to methicillin. MRSA by definition is any strain of *S. aureus* that has developed resistance to beta-lactam antibiotics which include beta-lactam stable formulations such as methicillin, oxacillin, flucloxacillin, nafcillin and cephalosporin. These MRSA strains are often responsible for several difficult to treat infections in humans (13). Knowledge of epidemiology of bacterial infections is very important for appropriate decision-making in the treatment of infections, such as septicemia, wound infections, and postsurgical infections. Iroha *et al.* (14) investigated cases among neonates in Lagos, Nigeria, in a prospective study. The incidence was 18 per 1,000 live births. *S. aureus* (37.4%) was the predominant etiologic pathogen among the bacteria. Another study investigated the bacteriology of nonsurgical wound infections in Ibadan. *S. aureus* (38%) was the predominant pathogen, followed by gram-negative bacteria (7 to 19% each). High rates of antibiotic resistance were recorded among these isolates (15).

The prevalence of MRSA in our study was higher (57.3%) compared to those in previous studies in South-western Nigeria. However, it should be considered that the presence of *themecA* gene, which is the "gold standard" for determining methicillin-resistance, was not investigated in some of these studies.

A recent multicentre study in South-western Nigeria confirmed resistance to methicillin by the detection of *themecA* gene by PCR and reported a lower prevalence rate of 14% (16). Obasuyi also used molecular techniques and reported the prevalence of 11% MRSA from clinical samples with two PFGE types (17).

Despite the high MRSA rate in our study, it is evident that multidrug resistant strains occurred frequently in South-western Nigeria. However, the MRSA isolates were predominantly associated with infections (57.3%), since all isolates were from clinical samples as also observed in other studies elsewhere (27). Nevertheless, the prevalence of MRSA was higher in this study than that Taiwo *et al.* (18) which showed the rate of 29%.

A major problem in the treatment of *S. aureus* infections is the multidrug resistance pattern of the pathogen to a number of antibiotics. In the last few years, understanding of the genetic basis for methicillin resistance has advanced significantly. Multi-resistant MRSA have been reported to be relatively high in African countries including Morocco, Kenya and Cameroun (19). A majority of the MRSA in our study showed multidrug resistance (83.7%). The misuse and misapplication of many antimicrobial agents in many parts of Nigeria may contribute to the high MRSA rate in this community. This poses a significant difficulty in antimicrobial agent choice for patients with this variety of infections which calls for periodic

surveillance of nosocomial infections due to *S. aureus* and other important bacterial pathogen in order to minimize microbial transmission.

CONCLUSION Effort must therefore be put in place at control measures that should include a renewed

awareness, isolation of MRSA infected patients in hospitals and multidrug resistance surveillance and enforcement of empiric use of antimicrobial agents to stem the tide of MRSA

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