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METHICILLIN RESISTANCE IN STAPHYLOCOCCAL ISOLATES FROM CLINICAL AND ASYMPTOMATIC BACTERIURIA SPECIMENS: IMPLICATIONS FOR INFECTION CONTROL

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The study assessed the importance of *Staphylococcus aureus* as a urinary pathogen and the incidence of multidrug resistant (MDR), methicillin-resistant *Staphylococcus aureus* (MRSA). A total of 86 staphylococcal isolates made up of 50 clinical isolates from urine samples submitted to the Medical Microbiology Laboratory of Ahmadu Bello University Teaching Hospital and 36 asymptomatic bacteriuria isolates from urine samples of 'healthy' volunteers within the university community were tested for their susceptibility to various antibiotics and production of -lactamase enzyme. A total of 27 isolates (31.4%) were methicillin resistant, with 12(44.4%) being methicillin resistant coagulase-negative staphylococci (MRCNS). Majority of the isolates tested were resistant to the cheap, readily available brand-spectrum antibiotics; ampicllin, amoxicillin, chloramphenicol, tetracycline and penicillin G. All the isolates were resistant to three or more of the antimicrobial agents tested. A total of 14/50 (28%) of the clinical isolates and 17/36 (47.2%) of the 'community' isolates from healthy volunteers were resistant to 7 or more of the antimicrobial agents tested. Analysis of the multiple antibiotic resistance (MAR) index of isolates and the production of -lactamase enzyme showed that 56 isolates representing 65.1% of the total number tested had an MAR index of 0.5 and above indicating that they probably originated from an environment where antibiotics are frequently used. The implication of these findings for instituting effective control measures aimed at reducing the pool of antibiotic-resistant organisms is discussed.

Key words: Methicillin-resistant, staphylococcus aureus, asymptomatic bacteriuria, infection control

INTRODUCTION

Antimicrobial resistance is a well recongnised problem worldwide (1, 2). The resistance organisms have however been associated primarily with hospitals, **especially** in intensive care units (1). The ubiquitous occurrence of staphylococci ensures that man is constantly exposed to this group of microorganisms, thus infection of various parts of the body caused by staphylococci are very common (3,4).

Staphylococcus aureus continues to be a major cause of community acquired and health-care related infections around the world (5,6). The emergences of high level of penicillin resistance followed by the development and spread of strains resistance to the semi-synthetic penicillins (methicillin, nafcillin and oxacillin), macrolides, tetracyclines and aminoglycoside have made the theraphy of staphylococcal disease a global challenge (6,7,8).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a virulent organism that causes significant mortality and morbidility, especially to patients in critical care areas (9). MRSA can (and does in some cases) also contribute to an increased length of hospital stay and healthcare costs. Infections with methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococci (MRCNS), have been widely reported. Those infections were initially confined to hospitals and nursing homes especially in intensive care units where a combination of debilitated patients, invasive technology, and high antimicrobial use facilitates infections by multi-drugs resistant staphylococci, enterobacteria resistant to third-generation cephalosporins and imipenem resistant non-fermentative bacteria (10). However, cases of community-acquired MRSA have been reported, primarily in persons with history of injection drug use and other high-risk patients (11).

Recently, community-acquired MRSA have been described in both adults and children who did not have extensive exposure to hospitals or apparent risk factors (12,13,14,15,16). Antimicrobial resistance often leads to therapeutic failure of empirical therapy; therefore, knowledge of the local prevalence of pathogens and their antimicrobial sensitivity patterns is essential for clinicians in their routine work (17). Effective antibiotic therapy in developing countries is severely limited by the large reservoir of antibiotic resistant bacteria that exist within their population. The healthy members of any community represent its largest reservoir of bacteria resistant to antimicrobial agents (18).

The increase in antimicrobial resistance is creating a lot of problems. These have focused attention upon measures for fighting resistance, foremost of which is susceptibility surveillance (19). The rapidity of emergence of multiple antibiotics resistant organisms is not being reflected by the same rate of development of new antimicrobial agents. It is therefore conceivable that patients with serious infections will soon no longer be treatable with currently available antimicrobial agents (20).

Before instituting control measures that will be appreciated by all healthcare professionals, there must be scientific data to ascertain the extent of the problem posed by multi-drug resistant organisms like the MRSA to the outcome of antimicrobial chemotherapy in the hospital and the immediate community. Unfortunately, surveillance studies

on the epidemiology of MRSA and their antimicrobial susceptibility patterns are lacking in this environment.

This study aimed at assessing the importance of *Staphylococcus aureus* as a urinary pathogen determine the incidence of multi-drug resistant, methicillin-resistant *Staphylococcus aureus* (MRSA) in clinical isolates from urine in a University Teaching Hospital and compare these with isolates from 'healthy' individuals within the university community. The implications for instituting effective control measures that can reduce the pool of antibiotic-resistant organisms within healthy members of the community and in the hospital setting are discussed.

MATERIALS AND METHODS

Bacteriology

Staphylococcal isolates obtained from urine sample submitted to the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, and 'healthy' student volunteers from Ahmadu Bello University were analysed.

The isolates were characterized using established methods, which included colonial morphology, Gram stain characteristics, ability to produce the enzyme peroxidase, coagulase and the presence of heat stable DNAse activity to separate the *Staphylococcus aureus* strains from the coagulase-negative staphylococci (CNS). A standard *Staphylococcus aureus* strain ATCC 13709 was obtained from the National Institute of Pharmaceutical Research and Development, Abuja, Nigeria.

Chemicals and media

The media used were Nutrient Broth (NB), Nutrient (NA) and Mannitol Salt Agar (MSA) all from Oxoid. The chemicals include hydrogen peroxide (3%), deoxyribonucleic acid, sodium chloride, starch and iodine solution.

Antimicrobial sensitivity testing

The susceptibility pattern of the isolates to the following antibiotics was determined; Ampicillin 25 µg, Chloramphenicol 20 µg, Cloxacillin 10 µg, Erythromycin 10 µg, Gentamcin µg, Penicillin G 1.5 iu, Streptomycin 10 µg, Amoxycillin 25 µg, Ciprofloxacin 5 µg and Methicillin 5 µg, using the modified Kirby Bauer diffusion technique (21). The isolates were grown overnight in nutrient broth and the inocula spread on the surface of the previously prepared sterile nutrient agar plates by flooding with 2mls of the standardized suspension. Excess were drained off and allowed to dry in a warm incubator for about 15-20 minutes. Using sterile forceps, multiantibiotic discs were placed on

the dried nutrient agar plate and left at room temperature for about 25minutes to allow the antibiotics to diffuse in the agar medium. Similar treatment was extended to the standard *Staphylococcus aureus* ATCC 13709. All the plates were incubated at 37° C for 24 hours in inverted position. Thereafter, the diameter of the zones of inhibition of the isolates and the standard *Staphylococcus aureus* were measured to the nearest millimeter.

Determination of Methicillin sensitivity

Nutrient agar medium containing 5% w/v sodium chloride (22) was prepared, distributed into 20ml aliquots and sterilized at 121° C for 15 minutes. Overnight cultures of the isolates were used to flood the surfaces of the prepared agar media, drained and allowed to dry. Methicillin discs (containing 5µg of methicillin) were placed on the dried agar plate and treated as previously described above, but incubation was at 35° C. The diameter of the zones of inhibition was similarly determined.

Test for β-lactamase production

Suspensions of the isolates were prepared in triplicates by emulsifying bacterial colonies (from an overnight nutrient agar culture) with sterile loops in 0.5 ml of phosphate buffer solution containing 0.06 mg/ml (10,000 units/ml) of Penicillin G. As control, cell suspension of the standard typed culture of *Staphylococcus aureus* (ATCC 13709) was similarly set-up. They were incubated at room temperature for at least 1 hour. Thereafter, 2 drops of freshly prepared 1% aqueous starch solution were added to each bacterial suspension and shaken. To this was added 1 drop of iodine solution and allowed to stand for 10 minutes at room temperature. β-lactamase producing organisms changed the colour of the reaction mixture from blue-black to colourless within the 10 minutes.

Determination of multiple antibiotic resistance (MAR) index

The MAR index was determined for each isolate by dividing the number of antibiotics to which the isolates is resistant by the total number of antibiotics tested (23,24).

$$\text{MAR index} = \frac{\text{Number to which isolate is resistant}}{\text{Total number of antibiotics tested}}$$

RESULTS

Of the staphylococcal isolates, 50 were clinical isolates from urine samples submitted to the Department of Medical Microbiology, Ahmadu Bello Teaching Hospital, while 36 were "community" isolates form urine samples of 'healthy' volunteers within the university community. A total of 27 isolates (31.4%) were

methicillin resistant, with 12 (44.4%) being MRSA, while 15 (55.6%) were MRCNS. Out of the 59 methicillin-sensitive staphylococcal isolates, 40 (67.8%) were MSSA while 19 (32.2%) were MSCNS. Figure 1 shows the proportion of the staphylococcal isolates resistant to various antibiotics.

The MAR index of isolates by the proportion, that are β-lactamase positive and methicillin-resistant is shown on Table 1. A breakdown of the analysis of the MAR index of the 'community' and clinical isolates is shown in Table 2.

All the isolates were resistant to three or more antibiotics, while 17/36 (47.2%) 'community' isolates and 14/50 (28%) clinical isolates showed multi-drug resistance to seven or more of the antibiotics tested. Figure 1 shows the susceptibility patterns of staphylococcal to different antibiotics. Table 1 shows multiple antibiotic resistance (MAR) index of *Staphylococcus aureus* isolates, with the proportions that are β-lactamase positive and methicillin-resistant. Table 2 shows the analysis of MAR index of the clinical and asymptomatic bacteruria (community) isolates.

Figure 1: Susceptibility patterns of Staphylococcal isolates to different antibiotics

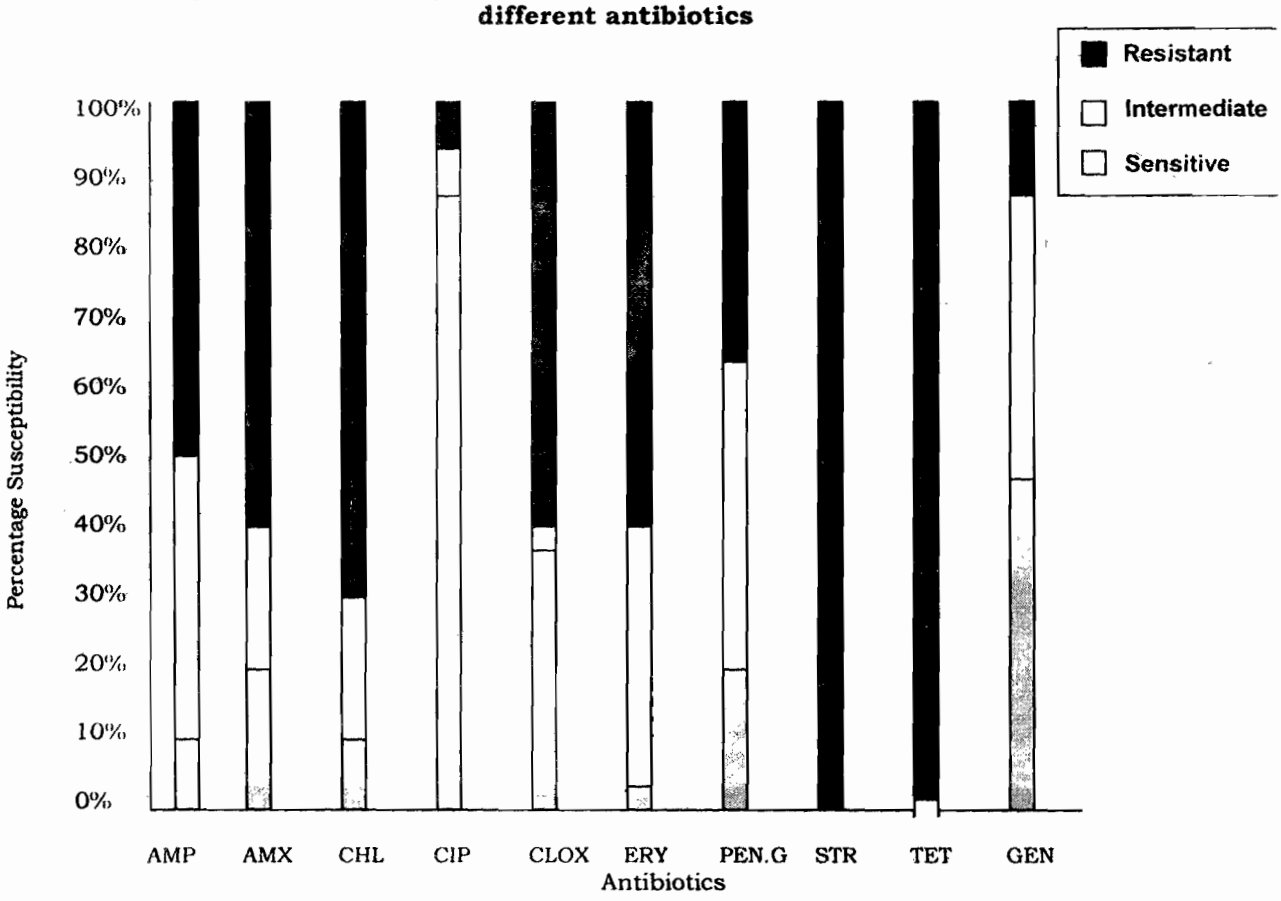

Table 1: Multiple antibiotic resistance (MAR) index of *Staphylococcus aureus* isolates, with the proportions that are β-lactamase positive and methicillin-resistant.

MAR index	No. of isolates (%)	β-lactamase +ve (%)	MR (%)
0.3	19(22.1)	3(15.8)	2(10.5)
0.4	10(11.6)	0(0.0)	1(10.0)
0.5	12(14.0)	3(25.0)	1(8.3)
0.6	12(14.0)	7(58.3)	3(25.0)
0.7	11(12.7)	7(43.6)	6(54.5)
0.8	15(17.4)	10(66.7)	8(53.3)
0.9	6(7.0)	3(50.0)	6(100.0)
1.0	1(1.2)	1(100.0)	0(0.0)

Table 2: Analysis of MAR index of the clinical and asymptomatic bacteruria (community) isolates number of

MAR index	Community isolates	Clinical isolates
0.3	3	17
0.4	3	7
0.5	4	8
0.6	8	4
0.7	6	5
0.8	4	7
0.9	4	2
1.0	1	0
TOTAL	**36**	**50**

DISCUSSION

Nosocomial infection caused by multi-resistant organisms in developing countries respresent a major public health problem that is not universally recognized (25). Results from various studies in the past did not identify *Staphylococcus aureus* as an important urinary pathogen (3,26,27), rather there has been a focus on the CNS identified as a mjor cause of infections associated with prosthetic implants and medical devices (28) and urinary tract infection, particularly in young sexually active women (29,30). The 72.4% prevalence of *Staphylococcus aureus* in this study points to the increasing important of this organism as a urinary

pathogen and a common isolate in asymptomatic bacteriuia in this environment.

Majority of the isolates tested were resistant to the cheap, readily available broad- spectrum antibiotics; Ampicillin (89.3%), Amoxycillin (83.3%), Chloramphenicol (89.3%), Tetracycline (98.8%) and Penicillin G (83.3%). This result is consistent with the observation that clinical staphylococcal isolates are resistant to a large number of commonly prescribed antimicrobial agents (30).

The level of multi-drug resistance exhibited by the staphylococcal isolates in this study is alarming. All the isolates were resistant to three or more of the antimicrobial agent tested. The MDR strains came from both clinical and 'community' isolates. A total of 14/50 (28%) of the clinical isolates and 17/36 (47.2%) of 'community' isolates form healthy volunteers wee resistant to seven or more of the antimicrobial agents tested. The high percentage of isolates form 'healthy' individual showing high MDR goes to confirm the assertion that the healthy members of the community represent its largest reservoir of bacterial resistant to antimicrobial agents (18,31). MAR index higher that 0.2 has been said to be an indication of isolates originating from an environment where antibiotic were often used (23,24).

Since all the isolates were not from the hospital environment where antibiotic are often used but also from urine of asymptomatic 'healthy' volunteers in the university community, this observation goes to confirm the widespread abuse/misuse of antibiotics in this community. Administration of antibiotics often permits the selection and overgrowth of multiply resistant organisms (32). The selective pressures favouring resistant strains are known to arise form misuse and overuse of antimicrobials (notably extended spectrum cephalosporins) increased numbers of immunocompromised hosts, lapses in infection control (where they exist), increased use of invasive procedures and devices, and widespread use of antibiotic in agriculture and animal husbandary (33).

It has been documented that resistance properties are easily transferred between organisms of the same or different genera through the agency of plasmid. Evidence of transfer of high-level resistance to gentamicin, tobramycin and kanamycin between staphylococi of the same and different species by filter mating also exists (34). Restriction endonuclease analysis of the plasmids from five isolates of

Staphylococcus epidermidis has also supported the hypothesis that plasmid transfers tween the two species occur in nature (35). Transfer of such resistance determinants may have been responsible for the high level of MDR encountered in this study.

A breakdown of the number of isolates with a particular MAR index and proportion that are β-lactamase-positive showed that 56 isolates representing 65.1% of the total number tested, had an MAR i of 0.5 and above, while 29 isolates (33.7%) had MAR index less than 0.5. This is a relative indication of the susceptibility of the isolates to the test antibiotics. One isolate that was resistant to all the 10 antibiotics tested was isolated from the urine of a 'healthy' volunteer in the community. The isolate produced β-lactamase, was coagulase-positive but sensitive to methicillin.

Multi-drug resistant *Staphylococcus aureus* have been known to produce β-lactamase in greater amounts than strains that are fully sensitive to antibiotic or resistant to only to penicillin (22). Contrary to the findings from other studies (4,36,37), bacterial resistance to ciprofloxacin (a fluoroquinolone) as high as 13.1% was encountered in this study. The increasing resistance to such a new and expensive, reserve drug is probably an indication of the increasing level of availability, misuse and overuse.

The tremendous therapeutic advantage afforded by introduction of new antibiotic is always threatened by the emergence of increasingly resistance strains of microbes (33). Introduction of new antibiotic are essential, but their useful life will be enhanced only if used wisely and sparingly (38). The ever-present danger of individuals contracting infections in this community makes it imperative that measures aimed at reducing the pool of antibiotic resistant organisms existing within the healthy members of the community be instituted within delay.

In concert with improved prescribing habits, efforts to identify and isolate resistant organisms that can be introduced into healthcare setting from outside institutions are essential. Evidence from various studies have shown that, surveillance when used to guide polices on antibiotic use and infection control, can be helpful to control the development and spread of antimicrobial resistance within the hospital setting and the community at large (19,39,40).

We agree that persistence would be required in influencing the behaviour of healthcare professionals and to maintain optimal infection control policies and procedures within the hospital

and the community at large. In the meantime, it is highly desirable to continuously monitor the antibiotic resistance situation so as to maximize the possibility of administering an effective antimicrobial agent whenever there is need to do so.

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