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PATTERN OF RESISTANCE TO VANCOMYCIN AND OTHER ANTIMICROBIAL AGENTS IN STAPHYLOCOCCAL ISOLATES IN A UNIVERSITY TEACHING HOSPITAL

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Multidrug resistance has been reported in clinical isolates of both coagulase-negative staphylococci (CoNS) and *Staphylococcus aureus* that are most often resistant to oxacillin/methicillin. Vancomycin, a glycopeptide is the drug of choice for infections caused by such multidrug resistant strains. This study determined the pattern of resistance to vancomycin and other antimicrobial agents in staphylococcal isolates from a University Teaching Hospital. Staphylococcal isolates from clinical specimens submitted to the diagnostic medical microbiology laboratory of the Ahmadu Bello University Teaching Hospital, Zaria (over a three-month period) were characterized using standard microbiological procedures and their susceptibility to vancomycin and other commonly used antimicrobial agents determined by Kirby-Bauer-NCCLS modified disc diffusion technique. A total of 56 of the 97 (57.7%) staphylococcal isolates characterized were resistant to vancomycin 30µg, showing a zone of inhibition less than 15mm. Most of these isolates were from urine (27.3%), wound (21.8%) and pleural aspirate (12.8%). The 56 staphylococcal isolates were made up of 75% (41/56) *Staphylococcus aureus* and 25% (14/56) coagulase-negative staphylococci. Majority of the isolates, 60.7% (34/56) produced β-lactamase enzyme. Resistance pattern to other antimicrobial agents was benzyl penicillin G (92.9%); tetracycline (69.6%); cefuroxime (60%); chloramphenicol (54.5%); oxacillin (49.1%); erythromycin (35.7%); gentamicin (25%) and ciprofloxacin (16.1%). Analysis of the multiple antibiotic resistance index (MARI) showed that majority (91.1%) were resistant to 3 to 7 of the other antimicrobial agents tested. No isolate was resistant to all the tested antimicrobial agents. A very high proportion of the staphylococcal isolates were resistant to vancomycin, a glycopeptide that is not commonly used in this environment. Ciprofloxacin and gentamicin appear to be the only agents that will be effective in treating infections by these isolates. The high proportion of isolates with MARI of 0.3 and above, suggest that the isolates originated from an environment where antibiotics are often used. There is need for constant, on-going antimicrobial resistance surveillance in important and commonly isolated clinically significant pathogens to form the basis for developing and implementing measures that will reduce the burden of antimicrobial resistance.

Keywords: vancomycin, methicillin resistance, *Staphylococcus aureus*, coagulase-negative staphylococci, antimicrobial agents

INTRODUCTION

Antimicrobial resistance is fast assuming an alarming proportion in many bacterial populations. The therapy of infectious diseases caused by bacteria resistant to multiple antimicrobial agents has emerged as one of the greatest challenges facing clinicians worldwide (1). The staphylococci present a special problem; with the coagulase-negative staphylococci (CoNS) associated with infections at the site of an indwelling catheter or cannula, cardiac and orthopaedic surgery involving the insertion of prosthetic devices and common cause of urinary tract infections (2). The coagulase-positive, *Staphylococcus aureus* is perhaps the pathogen of greatest concern because of its virulence (3), its ability to cause a diverse array of life threatening infections, and its capacity to adapt to different environmental conditions (3,4,5).

The first type of resistance to emerge in the staphylococci was the production of β-lactamase; enzymes that destroy the antibiotic by hydrolysing the β-lactam ring (6). Resistance to the β-lactamase stable penicillins (methicillin, oxacillin, nafcillin) is due to the production of altered cell-wall synthesis enzymes, the penicillin-binding proteins (PBPs) that are encoded on the *mec A* gene and function by preventing the binding of penicillin (6). Not many attempts have been made to document the prevalence of methicillin-resistant staphylococci in this environment (7, 8).

Vancomycin, a glycopeptide antibiotic was introduced into clinical practice in 1958 for the treatment of Gram-positive bacteria (4). The emergence and spread of vancomycin-resistant enterococci (VRE), the discovery that the genetic material responsible for high levels of vancomycin resistance in enterococci could be transferred *in vitro* to *S. aureus* was a great public health concern for many years (6,9). The development of very low levels of vancomycin resistance was first reported in CoNS in 1979 (10) and 1983 (11), while the first reported instance of vancomycin resistance in *S. aureus* occurred in Japan in 1996 (12). There have since been many other reported cases from other countries (10, 13-16). The mechanism of this resistance was observed to be distinct from that which mediates .vancomycin resistance in enterococci but appears to occur in a stepwise fashion as a result of long term, nearly constant exposure to vancomycin or glycopeptide (6) The glycopeptides (vancomycin or teicoplanin) are not used in this environment.

The purpose of this study was to determine the pattern of resistance in staphylococcal isolates from clinical specimens at the Ahmadu Bello University Teaching Hospital to vancomycin and other antimicrobial agents as a guide in assessing the need for the development of specific guidelines on the surveillance of this group of pathogens.

MATERIALS AND METHODS

Bacteriology

Staphylococcal isolates from all specimens submitted to the diagnostic medical microbiology laboratory of the Ahmadu Bello University Teaching Hospital, Zaria over a three-month period, were analyzed. The isolates were characterized using established microbiological methods, which included colonial morphology, Gram-stain characteristics, ability to produce enzyme peroxidase and coagulase to separate the *S. aureus* strains from the coagulase-negative staphylococci (17).

Antimicrobial susceptibility testing

The antimicrobial susceptibility pattern of the isolates was determined using the Kirby-Bauer-NCCLS modified disc diffusion technique (18). All the strains were tested for their sensitivity to the following antibiotics; gentamicin 10µg, cefuroxime 30µg, chloramphenicol 30µg, erythromycin 15µg, tetracycline 30µg, vancomycin 30µg, benzyl penicillin G 10i u, oxacillin 1µg and ciprofloxacin 5µg (all from bio Mérieus sa 69280 Marcy l'Etoile-France). The zones of inhibition were recorded and the isolates classified as 'resistant'; 'intermediate' or 'sensitive' based on the interpretative chart updated according to the current NCCLS standards (18).

Test for β-lactamase production

Suspensions of the isolates were prepared by emulsifying bacterial colonies (from overnight nutrient agar culture) with sterile loops in 0.5ml of phosphate buffer solution containing 0.06mg/ml (10,000units/ml) of benzyl penicillin (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 1hour. Thereafter, 2 drops of freshly prepared 1% aqueous starch solution were added to each bacterial suspension and shaken. Then, 1 drop of iodine solution were added and allowed to stand for 10minutes 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 MAR index

The multiple antibiotic resistance (MAR) index was determined for each isolate by dividing the number of antibiotics to which the isolate is resistant to by the total number of antibiotics tested (19, 20).

RESULTS

The distribution of the vancomycin-resistant staphylococcal isolates from various specimens is shown in Fig.1. A total of 56/97 (57.7%) of the staphylococcal isolates were resistant to vancomycin 30µg, showing a zone of inhibition less than 15mm (18). The vancomycin-resistant isolates were made up of *Staphylococcus aureus* 75% (41/56) and coagulase-negative staphylococci 25% (14/56). The resistance pattern of the vancomycin-resistant isolates to other antimicrobial agents is shown in Table 1.

Table 1: Antimicrobial Susceptibility of Vancomycin-Resistant Staphylococcal Isolates

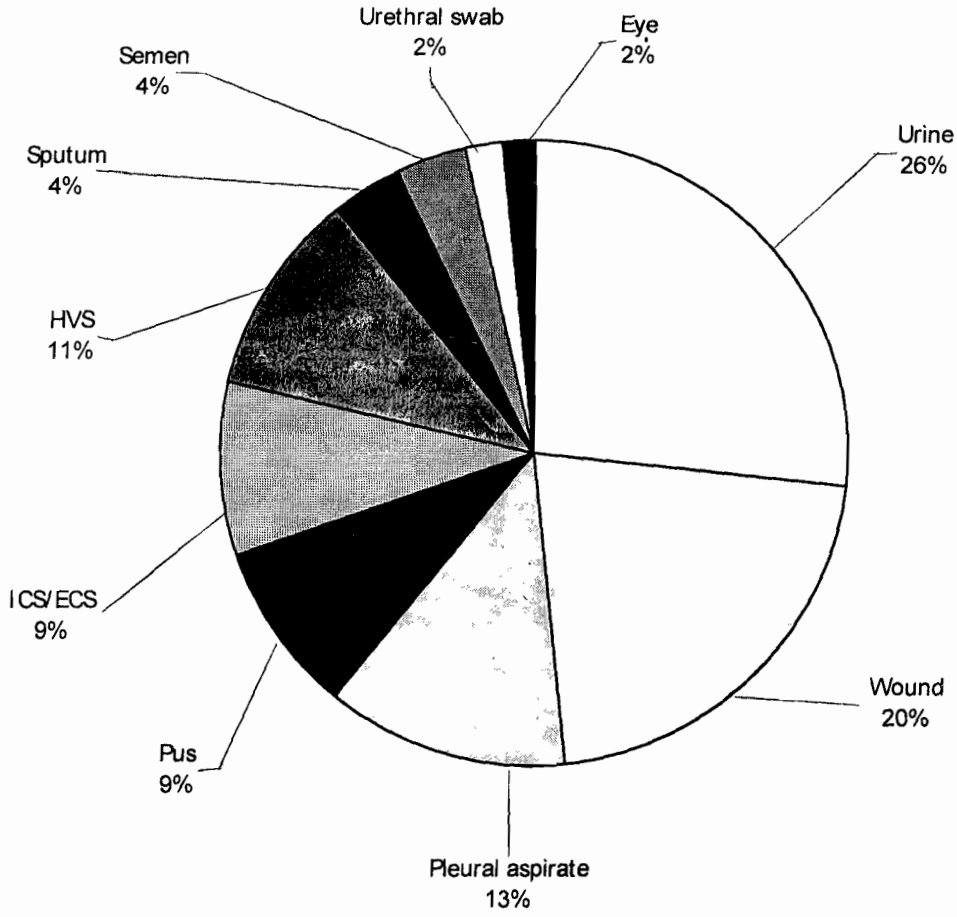
Antimicrobial agent	Disc Potency	% Resistant
Gentamicin	10µg	25
Cefuroxime	30µg	60
Chloramphenicol	30µg	54.5
Erythromycin	15µg	35.7
Tetracycline	30µg	69.6
Penicillin G	10i.u	92.9
Ciprofloxacin	5µg	16.1
Oxacillin	1µg	48.2

Only 27/56 (48.2%) of these vancomycin-resistant staphylococcal isolates were either oxacillin-resistant *Staphylococcus aureus* (ORSA) or oxacillin-resistant coagulase-negative staphylococcus (ORCoNS). Analysis of the multiple antibiotic resistance index (MARI) of the isolates shows that 91.1% were resistant to 3 or more antimicrobial agents (Table 2).

Table 2: Multiple Antibiotic Resistance Index of Vancomycin-Resistant Staphylococcal Isolates

MAR Index	No. of Isolates	Percentage
0.1	2	3.57
0.2	3	5.36
0.3	9	16.07
0.4	7	12.50
0.6	10	17.86
0.7	11	19.64
0.8	8	14.29
0.9	6	10.71

Fig. 1: Distribution of Vancomycin-Resistant Staphylococcal Isolates in Clinical Specimens



DISCUSSION

The level of vancomycin resistance in the staphylococcal isolates in this study was 57.7%. A previous study on the antibiogram and β-lactamase production of *Staphylococcus aureus* isolates from different clinical specimens (21) reported 100% sensitivity of the 73 isolates to vancomycin. Historically, the dramatic increase in the use of vancomycin in the treatment of infections caused by methicillin-resistant staphylococci (both coagulase-positive and coagulase-negative), *Clostridium difficile*, and the enterococcal infections preceded the emergence of vancomycin-resistant staphylococci internationally (5, 22). There is no such history of vancomycin use in this environment.

Vancomycin resistance can be difficult to detect in the clinical laboratory (4). Tenover *et al* (23) reported that the disc diffusion sensitivity testing using standard 30μg vancomycin disc frequently misclassify intermediately susceptible isolates as fully

susceptible. In a study (24), 75% of microbiology laboratories around the world actually misreported a glycopeptide-intermediate strain of *S. epidermidis* as susceptible based on the results of disc diffusion testing. The likelihood in this present study is the under detection of vancomycin resistance.

All strains of glycopeptide-resistant *Staphylococcus aureus* recovered to date are all oxacillin/methicillin resistant and were not clonal. Many of the patients had received vancomycin and had ORSA/MRSA infections (5, 6, 25). In this present study, only 48.2% of the isolates were either ORSA or ORCoNS. Since there was no perceived danger from ORSA or ORCoNS infections in this environment, isolates are not routinely tested for susceptibility to oxacillin/methicillin, therefore, there has been no need for the inclusion of vancomycin disc in sensitivity testing or on the hospital formulary and procurement by the hospital pharmacy.

Vancomycin resistant staphylococcal isolates in this study were resistant to 3 to 8 of the tested antimicrobial agents. This is consistent with the observation that all reported cases of glycopeptide-resistant *Staphylococcus aureus* (GRSA) cases have occurred in ORSA that are often resistant to wide variety of antibiotics (6). Most of the isolates showed a reasonably high sensitivity to ciprofloxacin (83.9%), gentamicin (75%) and erythromycin (64.3%). Given the paucity of cases, there are no formal recommendations for the treatment of infections with coagulase-negative staphylococci with reduced susceptibility to vancomycin (4), but in one of the reported cases, the infection was successfully treated with erythromycin (26). Interestingly also, vancomycin intermediate *Staphylococcus aureus* (VISA) isolates in the United States were all sensitive to trimethoprim-sulfamethoxazole and tetracycline, while tobramycin, rifampicin, gentamicin, doxycycline are some of the drugs that have been successfully used clinically (4, 11, 27-30).

β-lactam antibiotics have also been used in the treatment of VISA cases, once in combination with aminoglycoside (12) and vancomycin (11). Only 48.2% of the vancomycin-resistant isolates were resistant to oxacillin 1μg. Although the specific role played by penicillin-binding proteins (PBPs) in vancomycin resistance remains unclear (4), studies have demonstrated that the MIC to oxacillin of some VISA isolates decreased as the vancomycin MIC goes up (31, 32). This supports the *in vitro* findings that the mutated penicillin-binding protein, PBP2a or PBP2' responsible for methicillin/oxacillin resistance is down regulated in vancomycin-resistant isolates (33). The high level of susceptibility of the vancomycin-resistant staphylococcal isolates to ciprofloxacin and gentamicin (two drugs frequently prescribed in this environment) may account for why infections by any of these isolates have not constituted a noticeable clinical problem.

There is however the need for more clinical data to assess the clinical significance of the vancomycin resistance encountered in this study and their attributable mortality and morbidity. There is also the need for consistent, on-going antimicrobial resistance surveillance for important and commonly isolated clinically significant pathogens like the MRSA, VISA, VRSA, and VRCoNS to form the basis for

developing and implementing measures that can reduce the burden of antimicrobial resistance and prevent a probable impending public health problem.

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