PREVALENCE AND ANTI-BIOTIC SUSCEPTIBILITY PATTERN OF HIGH LEVEL AMINOGLYCOSIDE RESISTANCE AMONG GRAM POSITIVE ORGANISMS IN A TERTIARY CARE CENTRE

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ABSTRACT

Objective: The study is done to determine the prevalence and the antimicrobial susceptibility against the gram positive organisms with high level aminoglycosides resistance.

Methods: This is a prospective study involving the analysis of clinical samples for gram positive organisms like Streptococcus species, Enterococcus species and Staphylococcus species and determining their antimicrobial susceptibility pattern.

Results: From the collected clinical samples, all the gram positive organisms show resistance to gentamicin. Most of the Staphylococcus species showed a wide resistance to erythromycin followed by ceftriaxone and many Enterococcus species shows resistance to penicillin, erythromycin, ciprofloxacin.

Conclusion: The high level aminoglycoside resistant (HLAR) gram positive organisms showed susceptibility to few drugs that are prevalent in various clinical samples. Surveillance of antimicrobial resistance and sensitivity is essential in managing and controlling infection.

Keywords: Aminoglycoside, Staphylococcus, Enterococcus, Streptococcus, Antimicrobial susceptibility

INTRODUCTION

Aminoglycosides have been an important part of the antibiotics used since 1940s, which is obtained by screening of soil Actinomycetes for the elaboration of antimicrobial substance that yielded streptomycin [1]. Drugs that are classified under aminoglycosides are obtained from Streptomycisspp. (Drugs name ending with -mycin) neomycin, kanamycin, tobramycin or also derived from micromonosporaspp. (Drugs name ending with -micin) gentamicin, netilmicin, amikacin, sisomycin etc. Aminoglycosides exhibit concentration-dependent killing (CDK) and have prolonged post-antibiotic effects (PAE). That is on increasing the concentration of antibiotic, the rate of bactericidal action increases and on the other hand, on decreasing the concentration there is a persistent suppression of bacterial growth [2].

Aminoglycosides show their bactericidal activities against the aerobic organisms as they need oxygen for active transport through the inner membrane; therefore, these drugs are inactive against anaerobes. These acts by binding to the 30S ribosome and freeze the initiation, interfere with the polysome formation and causes misreading of mRNA code [3]. They act against the gram-negative bacteria and the gram-positive organisms as well. Aminoglycosides along with penicillin, show a synergistic effect as the penicillin, being cell wall inhibitor, enhances the transport of aminoglycosides [4].

Acquired drug resistance in a bacteria may be due to single-step mutation (streptomycin etc...) or multistep (erythromycin etc...). The high-level resistance to gentamicin among the clinical isolates of Enterococcus faecalis was first reported in 1978 [5]. The transfer of resistance against aminoglycosides is through conjugation [6]. And the mechanism through which it develops is due to the formation of inactivating enzymes that acetylate, phosphorylate or adenylate the aminoglycoside and also through decreasing the drug permeability due to loss of specific channels [7]. The importance of these observations are based on the fact that high level aminoglycoside resistance confabulates the resistance to bactericidal synergism between that aminoglycoside and cell wall active antibiotics [6, 8].

MATERIALS AND METHODS

Isolation and identification

Gram-positive organisms were isolated from various clinical samples from both in-patients and out-patients over a period of five months from January to May 2020 in the clinical laboratory of microbiology department, Saveetha Medical College and Hospital, Thandalam, Tamil Nadu. The samples from which the bacterial species isolated were exudates, urine, blood and respiratory samples and a routine Gram Staining was done. From these, the samples were inoculated on to blood agar and MacConkey agar plates and determining their antimicrobial susceptibility pattern.

Antimicrobial susceptibility testing

Kirby Bauer disk diffusion method was used to perform the antimicrobial susceptibility test as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2002) [10]. Individual isolates were inoculated on Mueller Hinton Agar plates at a concentration of 106 Colony Forming Units (CFU) by lawn culture method [11, 12]. Discs impregnated with antibiotics Ampicillin (10mcg), Ceftriaxone (30mcg), Cefoxitin (30mcg), Cefotaxime (25mcg), Ciprofloxacin (5mcg), Erythromycin (15mcg), Linezolid (30mcg), Norfloxacin (30mcg), Nitrofurantoin (300mcg), Penicillin (10mcg), Tetracycline (10mcg), Vancomycin (30mcg), High Level Gentamicin (120mcg) were placed. Plates were examined after 24h of incubation at 37 °C.

RESULTS

In this study, a total of 97 clinically relevant gram-positive cocci were identified. Colony characteristics on the agar plates and the
reactions observed from the biochemical tests performed for each isolates were noted [table 1]. The most prevalent gram-positive cocci isolated were *Staphylococcus* species followed by *Enterococcus* species and *Streptococcus* species.

### Table 1: Colony characteristics and general biochemical test reactions used for the isolates

| Staphylococcus species | Streptococcus species | Enterococcus species |
|------------------------|-----------------------|----------------------|
| **Gram staining**       | Gram-positive cocci in clusters | Gram-positive cocci in chains and pairs |
| On Blood Agar           | Colonies with narrow zones of beta haemolysis | Pin point colony with wide zone of beta hemolysis (*S. pyogenes*) or alpha hemolysis (*S. viridans*) |
| On Mac conkey agar Catalase | Pink colonies due to lactose fermentation | Granular turbidity with powdery deposit (S. pyogenes) |
| Slide coagulase         | Positive-Coarse clumps formed (Staphylococcus aureus) | Optochin sensitivity |
|                         | Negative-no clumps (Coagulase negative Staphylococcus) | Presence of zone of inhibition (S. pneumoniae) |
| Tube coagulase          | Growth is seen. | 6.5% NaCl |

The distribution of clinical samples, which includes blood, exudates, urine and respiratory samples from which these gram-positive cocci were isolated is given in table 2.

### Table 2: Distribution of various specimen type among the gram-positive bacteria

| Specimen type       | Number of isolates | Staphylococcus species | Streptococcus species | Enterococcus species | Total |
|---------------------|--------------------|------------------------|-----------------------|----------------------|-------|
| Blood               | 20                 | 1                      | 2                     | 23                   |
| Exudates            | 26                 | 1                      | 1                     | 28                   |
| Urine               | 16                 | -                      | 24                    | 40                   |
| Respiratory samples | 6                  | -                      | -                     | 96                   |
| **Total**           | **68 (70.1%)**     | **2 (2.1%)**           | **27 (27.8%)**        | **97**               |

Out of 97 clinical specimens, 71 were from in-patient (IP) samples and 26 were from out-patients (OP).

From table 2 and 3, it is interpreted that high-level aminoglycoside resistant gram-positive organisms like *Staphylococcus* species, which includes MRSA and CoNS were predominant in blood, exudate and in respiratory samples and least in the urine sample and accounts for 70.1% of total clinical isolates whereas *Enterococcus* species were predominant in the urine sample as compared with the blood and exudate and it accounts for 27.8% of the total clinical samples. *Streptococcus* species were the least of all and accounts for 2.1% of total clinical isolates. All the 27 Enterococcus spp and 2 Streptococcus spp showed High level Gentamicin resistance, whereas 56 out of 68 *Staphylococcus* spp showed resistance to HLG. The susceptibility test is conducted against various antibiotics out of which HLR organisms showed maximum resistance to erythromycin (68%) and ceftriaxone (66%) among *Staphylococcus* species and to ampicillin (56%) and penicillin (56%) among *Enterococcus* species (table 3). The *Streptococcus* species were resistant to the antibiotics like ampicillin, erythromycin, penicillin, cotrimoxazole and *Enterococcus* spp showed resistance towards ampicillin, few cephalosporins, norfloxacin, penicillin, tetracycline whereas, the *Staphylococcus* shows resistance to almost all of them. There was a notable range of cefoxitin resistance among the HLR *Staphylococcus* species that can be either Methicillin-Resistant *Staphylococcus aureus* (MRSA) or Methicillin Resistant Coagulase negative *Staphylococcus* spp. (MR CoNS).

There were 12 *Staphylococcus* species which were found out to be susceptible for High-level Gentamicin. All these 12 isolates were susceptible to vancomycin, linezolid, cotrimoxazole and ceftriaxone. The susceptibility test is conducted against various antibiotics out of which HLR organisms showed maximum resistance to erythromycin (68%) and ceftriaxone (66%) among *Staphylococcus* species and to ampicillin (56%) and penicillin (56%) among *Enterococcus* species (table 3). The *Streptococcus* species were resistant to the antibiotics like ampicillin, erythromycin, penicillin, cotrimoxazole and *Enterococcus* spp showed resistance towards ampicillin, few cephalosporins, norfloxacin, penicillin, tetracycline whereas, the *Staphylococcus* shows resistance to almost all of them. There was a notable range of cefoxitin resistance among the HLR *Staphylococcus* species that can be either Methicillin-Resistant *Staphylococcus aureus* (MRSA) or Methicillin Resistant Coagulase negative *Staphylococcus* spp. (MR CoNS).

### Table 3: Antimicrobial susceptibility pattern of HLR gram-positive bacteria

| Antibiotics used | Staphylococcus species | Streptococcus species | Enterococcus species |
|------------------|------------------------|-----------------------|----------------------|
| Ampicillin       | 11 (20%)               | 1 (50%)               | 15 (56%)             |
| Ceftriaxone      | 37 (69%)               | -                     | 3 (11%)              |
| Cefoxitin        | 24 (43%)               | -                     | -                    |
| Ciprofloxacin    | 12 (21%)               | -                     | 13 (48%)             |
| Cotrimoxazole    | 28 (50%)               | 1 (50%)               | 7 (26%)              |
| Erythromycin     | 38 (68%)               | 1 (50%)               | 13 (48%)             |
| Linezolid        | 2 (4%)                 | -                     | -                    |
| Norfloxacin      | 5 (9%)                 | -                     | 3 (11%)              |
| Nitrofurantoin   | 2 (4%)                 | -                     | 7 (26%)              |
| Penicillin       | 9 (16%)                | 1 (50%)               | 15 (56%)             |
| Tetracycline     | 6 (11%)                | -                     | 2 (7%)               |
| Vancomycin       | 2 (4%)                 | -                     | 12 (44%)             |

From table 3, we were able to interpret that most of the HLR *Staphylococcus* spp showed sensitivity for penicillin, linezolid, tetracycline and vancomycin. On the contrary, the majority of the HLR *Enterococcus* spp exhibited resistance to vancomycin, ampicillin and penicillin.
DISCUSSION

Over the last few years, we have witnessed different kinds of drug resistance emerging from various bacteria. The present study demonstrates the prevalence and antimicrobial susceptibility pattern of HLG resistance gram-positive organisms like *Streptococcus spp*, *Enterococcus spp*, *Staphylococcus spp* against various antibiotics. In our study, out of the total 97 isolates, the majority (73%) were recovered from hospitalized patients than the outpatients, similar to other studies [11].

Within the HLAR Staphylococcal isolates, higher resistance were towards erythromycin (68%), ceftriaxone (66%) and cotrimoxazole (50%), while it was more sensitive towards linezolid and vancomycin (96%) followed by penicillin (84%). Habeeb Khadri et al. [9] have shown a similar result with Staphylococcus spp ie., have shown resistance for cotrimoxazole but also shows more resistance for penicillin than our study and less percentage for vancomycin. From Mohanty S et al. [10], it was in accordance to our study that most of the *enterococcus spp* were resistant to penicillin, ciprofloxacin, erythramycin. According to our study, *Enterococcus* showed resistance to ampicillin too.

CONCLUSION

From this study, it is concluded that the HLG resistant gram-positive organisms are acquiring resistance to various other antibiotics too and is still sensitive to some, which varies with different bacteria. This study emphasizes the need for HLAR screening in patients suffering from various infections. Routine HLAR screening can help the clinicians in limiting the spread of resistance.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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