

Aminoglycoside resistance in enterococci isolated from paediatric septicaemia in a tertiary care hospital in north India

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Background & objectives: Enterococci are important nosocomial agents and serious infections caused by them are often treated with a combination of cell wall inhibitor and aminoglycoside. However, the presence of high level aminoglycoside resistance in these isolates makes this treatment combination ineffective. The prevalence of such isolates in a tertiary care set up has important diagnostic and therapeutic implications. The present study was carried out to find out the occurrence of high level aminoglycoside resistant isolates of enterococci in paediatric septicaemia cases in a tertiary care set up in north India.

Methods: Blood of paediatric cases with a clinical diagnosis of septicaemia was cultured to isolate and identify enterococci. Agar screen method was used to detect high level streptomycin and gentamicin resistance in these isolates. Vancomycin susceptibility of these isolates were determined as per the National Committee for Clinical Laboratory Standards (NCCLS) recommendations.

Results: Fifty one enterococcal strains were isolated from 21 neonates, nine infants and 21 children with a clinical diagnosis of septicaemia. Sixty eight per cent of these isolates had high level gentamicin resistance and forty three per cent had high level streptomycin resistance. All the isolates with high level streptomycin resistance also had high level gentamicin resistance. More than ninety five per cent of these isolates were sensitive to vancomycin.

Interpretation & conclusion: The occurrence of high level gentamicin and high level streptomycin resistance in enterococcal isolates in our set up was high. This would require routine testing of the enterococcal isolates for high level aminoglycoside resistance. Alternative treatment regimes need to be sought, besides prudent use of antibiotics.

Key words Enterococci - high level aminoglycoside resistance - tertiary care hospital

Over the years, enterococci have become increasingly resistant to antibiotics in terms of both the multiplicity of resistance and the level of resistance to particular drugs¹, and have emerged as important nosocomial agents due to their colonizing ability and multi drug resistance^{2,3}.

A common regime for treatment of serious enterococcal infections such as septicaemia is the

combination of cell wall inhibitors as penicillin, ampicillin or vancomycin with aminoglycosides such as streptomycin or gentamicin⁴. The addition of cell wall inhibitor agent helps in the penetration of the aminoglycoside into the bacterial cytoplasm making the intrinsically resistant organism as aminoglycoside sensitive. The presence of high level aminoglycoside resistance (HLAR) in enterococci defined as minimum inhibitory concentration of aminoglycoside for the isolate

>2000 µg/ml, makes the synergism of cell wall inhibitor and aminoglycoside ineffective⁵. Among the many mechanisms for the resistance in these isolates, one is the secretion of enzymes; which inactivate the aminoglycoside by different mechanisms as adenylation and phosphorylation^{5,6}.

There is limited information on the presence of HLAR enterococci isolated from paediatric septicaemia cases in a tertiary care set up. A high prevalence rate of these isolates would require routine screening for this characteristic and seeking of alternative regimes for the treatment of such cases. The present study was carried out to determine the occurrence of HLAR in enterococci isolated from paediatric septicaemia patients in a tertiary care set up in north India.

Material & Methods

Neonates (n=21) and children (n=30) including nine infants with clinical diagnosis of septicaemia admitted in Kalawati Saran Hospital, New Delhi, India between October, 2000 and June 2001 were included in the study. Blood was cultured using brain heart infusion broth (Hi-Media Laboratories, Mumbai, India). Subcultures were made from this broth to sheep blood agar at regular intervals for isolation of enterococci.

Enterococci were identified using standard methods based on gram staining, catalase reaction, bile aesculin, growth in 6.5 per cent NaCl and Sugar fermentation reactions⁷. Agar screen method was used to detect high level aminoglycoside resistance amongst the enterococcal isolates⁸. Briefly 5-6 colonies of the isolate were suspended in brain heart infusion broth and concentration adjusted to 0.5 McFarland standard (1.5×10^8 cfu/ml). It was subsequently diluted and 10 µl of the suspension was spotted on to the various quadrants of the Mueller Hinton plate such that each spot had 10^4 cfu. Acceptable growth on the control quadrants (*i.e.*, with no drug) indicated acceptable quality control. Presence of growth in the drug quadrant with streptomycin (2000 µg/ml) indicated the strain to be high level streptomycin resistant. Similarly growth in the quadrant with gentamicin indicated the strain to be high level gentamicin resistant. Growth in both the drug quadrants indicated high level resistance to both streptomycin and gentamicin. Vancomycin susceptibility

Table. Distribution of high level gentamicin and high level streptomycin resistance among the enterococcal isolates (n=51)

	No. of isolates (%)
HLGR (high level gentamicin resistance)	35 (68)
HLSR (high level streptomycin resistance)	22 (43)
HLGR + HLSR	22 (43)
HLGR (not associated with HLSR)	13 (23)

of the isolates was done and interpreted according to National Committee for Clinical Laboratory Standards (NCCLS) recommendations⁹.

Results

Fifty one enterococcal isolates were isolated from the paediatric septicaemia cases during the study period. Of these, 21 isolates were isolated from neonates, nine from infants and 21 from children. Thirty two of these isolates were from males.

There is a higher percentage positivity of the isolates having high level gentamicin resistance (68%) in comparison to the isolates having high level streptomycin resistance (43%). All the isolates that had high level streptomycin resistance also had high level gentamicin resistance but the opposite was not true (Table). Of the 35 high level gentamicin resistant isolates, 34 (97%) were vancomycin sensitive. Similarly a high level of vancomycin sensitivity (95%, 21 of 22) was observed in isolates with high level streptomycin resistance.

Discussion

High level aminoglycoside resistant enterococci often have plasmids which carry determinants encoding resistance to other antibiotics, besides limiting the option of using a combination of cell wall active antibiotics and aminoglycosides². This drug combination depends on the synergistic bactericidal activity between the two antibiotic groups, is often used empirically in serious infections⁵. A high occurrence of high level aminoglycoside resistance necessitates routine testing of the enterococcal isolates.

High level aminoglycoside resistant enterococci were first reported in France in 1979 and since then have been isolated from all the continents⁵. They comprise significant fraction of the clinical enterococcal isolates at some centres³. A study by Zervos and associates reported a prevalence of 55 per cent of high level gentamicin resistance in enterococci in an US centre³. Another study reported a prevalence rate of high level gentamicin resistance in enterococci varying from 1 to 49 per cent in the 27 European countries studied¹⁰.

In India (Nagpur), a study by Agarwal *et al*¹¹ reported a prevalence of high level gentamicin resistance in enterococci to be 7.8 per cent whereas high level streptomycin resistance was reported to be 24.7 per cent. The higher rates in the present study may be ascribed to the source of the isolates being from a tertiary care set up where chronic cases are prevalent and a wider usage of broad spectrum antibiotics occurs. The reason for the higher prevalence of high level streptomycin resistance in comparison to the high level gentamicin resistance in the enterococcal isolates in this study¹¹ was not clear. It may be related to greater usage of streptomycin in comparison to gentamicin¹². Majority of the enterococcal strains in our study were sensitive to vancomycin. Though the treatment regime with vancomycin would be effective but would not be practicable in our country as the drug is expensive and would be beyond the reach of most patients.

To conclude, the present study highlighted the importance of high occurrence of high level aminoglycoside resistant enterococci in our set up. This would necessitate routine testing of the isolates for high level aminoglycoside resistance. Alternative regimes in the management of enterococcal infection need to be evaluated.

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