



Vancomycin Resistant Enterococci in Preterm Neonates: A Rising Thunder in Intensive Care Units

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Abstract

Introduction: *Enterococci are one of the important global pathogens in neonatal septicemia considered as colonizers in past. Their gaining resistance has been highlighted mainly to Vancomycin and high level aminoglycosides.*

Material & Methods: *It was a retrospective study in which blood culture samples from neonatal intensive care unit received in the laboratory were considered. Processing of samples and identification of organisms were done as per standard guidelines.*

Results: *Enterococci accounted for 16 % of isolation rate. 78 % of them showed early onset septicemia with male predominance. Vancomycin resistance was observed in only one isolate (11 %) while high level aminoglycoside resistance in 80 % of them. Clinical history suggested low birth weight, prematurity, fever, catheterization and raised C- reactive protein levels.*

Conclusions: *The study shows upsurge of VRE and HLAR in neonatal septicemia with need of Active surveillance culture program for rapid identification of colonizers as the main cause of bacteremia.*

Keywords: *Enterococci, Neonatal septicemia, Vancomycin and high level aminoglycoside resistance.*

Introduction

Neonatal septicemia is a severe life-threatening bacteremia in which multiplying bacteria release toxins into the blood stream which increases production of cytokines causing clinical manifestations like fever, chills, tissue anoxia, reduced blood pressure, collapse etc. it can be early onset septicemia developing within a week of birth or late onset- after a week to a month. Enterobacteriaceae and Gram positive cocci

especially Staphylococci are the most common pathogenic group responsible for neonatal septicemia. But eventually Enterococci have evolved over past few decades from an intestinal commensal or only colonizer to one of the important and increasingly isolated pathogens in hospital acquired infections especially urinary tract infections, wound infections and septicemia. Their infection rate is gradually increasing as they have capacity to survive for long time globally

including inanimate objects like thermometers, stethoscopes and most importantly hands of health care professionals^(1,2). They are generally found in oral cavity, intestinal tract and female genital tract of humans^(3,4), so infection can be transmitted by various means such as during delivery or post partum. They generally cause late onset septicemia in newborn but incidence of early onset septicemia is also increasing⁽⁵⁾. As they are mainly nosocomial, chances of getting infection is more in immunocompromised patients such as in extremes of age like new born, children or elderly and more due to catheterization, irrational antibiotic usage, improper health care management etc. They are also difficult to treat as they have inherent resistance to many commonly used drugs like cephalosporins, low level aminoglycosides, trimethoprim-sulfamethoxazole and more^(6,7,8). In addition to that they have ability to develop different resistant genes which can give resistance not only to Enterococci but also to other group of organisms by gene transferring especially Staphylococci⁽⁹⁾. In this era of antibiotics, resistance to many routinely used drugs against Enterococci have been noticed like macrolides, glycopeptides especially Vancomycin, lipopeptides- Linezolid. Resistance to Vancomycin is of up most importance as this drug is commonly the preferred choice in combination with the other drugs due to synergistic effect. Resistance to this drug may be because of wide spread use of drugs which ultimately leads to adverse outcomes like prolonged hospital stay, high cost, increased morbidity and mortality. Further, high level aminoglycosides resistance is also increasing limiting the use of aminoglycosides and leaving behind only few therapeutic options. Moreover, VRE isolates are mainly multi drug resistant. Therefore, rapid identification of VRE with multi drug resistance should be done to control high infection rate. So this study was done to highlight gaining importance of multi drug resistant Enterococci in neonates which were considered previously non harmful colonizers only.

Material and Methods

It was a retrospective analytical study in tertiary care. All neonatal blood samples from neonatal intensive care unit received in laboratory for 6 months of period were considered in the study. Blood cultures were done as per standard guidelines on MacConkey agar, Blood agar with 5 % added sheep blood and Chocolate agar. Speciation of organism was done by gram staining, colony characteristics, bacitracin resistance and different biochemical reactions like bile esculin hydrolysis test, sugar fermentation test, arginine dehydrolase test and growth in 6.5 % NaCl. The antimicrobial susceptibility test for Vancomycin was tested by E-test- MIC (Minimum inhibitory concentration) method while other drugs by Kirby bauer disc diffusion method on Muller hinton agar as per CLSI guidelines 2016^(10,11).

Result

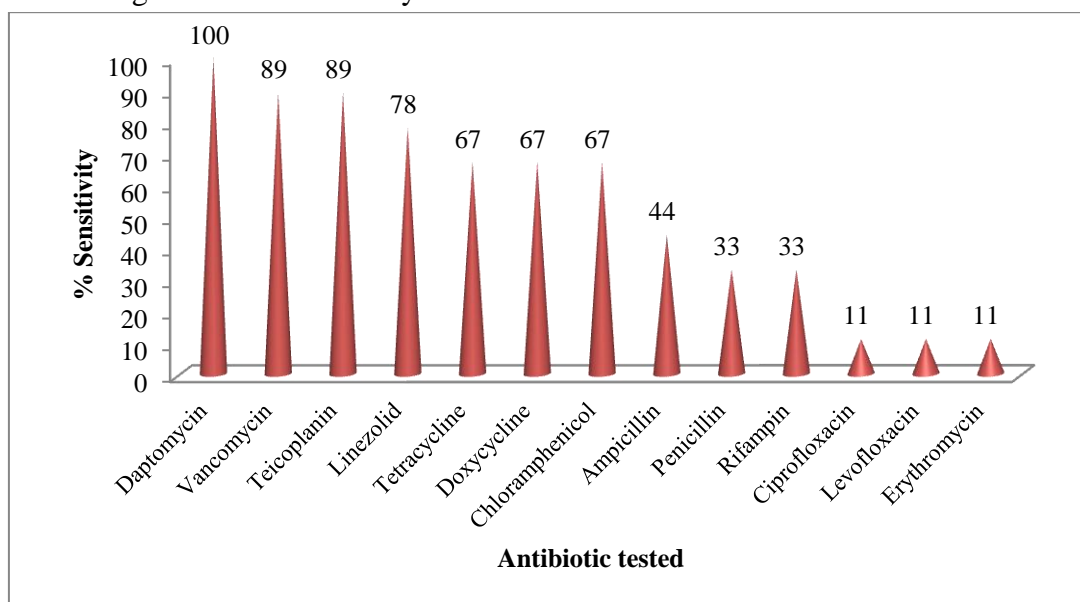
Total 145 samples were screened and from them Enterococci isolation accounted for 16 % with number of isolates being 9. Antibiotic sensitivity of Enterococci is shown in Table. It seems that most of the isolates were highly resistant to fluoroquinolones (Levofloxacin and Ciprofloxacin) and Macrolide (Erythromycin) showing only 10 % sensitivity. Sensitivity to Penicillin group of drugs ranged from 30 to 40 % only. Tetracyclines and Chloramphenicol showed around 70 % sensitivity. The Glycopeptide group showed 90 % of sensitivity while Lipopeptide (Daptomycin) was the only group showing complete sensitivity. The other group Oxazolidinone (Linezolid), the second most frequent group used for treatment was 80 % sensitive with 2 isolates showing resistance to it. One isolate (11 %) showed Vancomycin resistance (VRE) with MIC value >32 µg/ml. The VRE isolate was also resistant to Penicillin, Ampicillin, Erythromycin, Ciprofloxacin and Levofloxacin. It also showed High Level Gentamycin resistance. It was sensitive to Linezolid, Tetracycline group of drugs,

Daptomycin, Rifampin and Chloramphenicol. The drug Teicoplanin showed intermediate zones according to CLSI guidelines 2016. 78 % of Enterococcal isolates showed High Level Gentamycin resistance. Amongst Enterococcal isolates male predominance was observed with 78 % of isolates showing early onset septicemia (< 7 days of life) with peak at 3rd day of life, while only 22 % showing late onset septicemia (> 7 days of life). Growth of the Enterococci was predominantly (90 %) seen on 1st subculture than on 2nd subculture (10%).

Table 1: Antibiotic susceptibility of isolated Enterococci

No	Antibiotic tested	% Susceptibility of antibiotic
1	Daptomycin	100
2	Vancomycin	89
3	Teicoplanin	89
4	Linezolid	78
5	Tetracycline	67
6	Doxycycline	67
7	Chloramphenicol	67
8	Ampicillin	44
9	Penicillin	33
10	Rifampin	33
11	Ciprofloxacin	11
12	Levofloxacin	11
13	Erythromycin	11

Figure 1: Showing antibiotic sensitivity of Enterococcal isolates



Clinical correlation suggested that most of isolates were having preterm delivery with low birth weight, high grade fever, raised C- reactive protein, raised white blood cell counts and catheterization by one or the other ways such as vascular or urethral or enteral. The VRE isolate in addition showed respiratory distress syndrome leading to intubation of the neonate.

Discussion

Various clinical factors such as preterm delivery of newborn, low birth weight, immature immune system, respiratory distress syndrome, increase central line catheterization, increase parenteral

nutrition, improper hand hygiene, empirical antibiotics etc^(2,12) play important role in developing septicemia in first few days of life especially in these kind of new borns where their immunity is naturally suppressed or under developed. These factors could have been the reason for predominance of early onset septicemia in present study. The study showed 9 isolates of Enterococci with 1 isolate having Vancomycin resistance. The isolates in the study having one or more of these predisposing factors shows importance of them in developing septicemia. This shows *similar* results with *Paulo et al*⁽²⁾ and *Herrmann et al*⁽¹³⁾.

In present study prevalence of Enterococci in neonatal septicemia accounted for 16 % which is nearer with Gheibi *et al*⁽¹⁴⁾ that showed 22 % isolation, whereas that of High level Gentamycin resistance (HLGR) or High level Aminoglycoside resistance (HLAR) in neonatal septicemia was 78 %. This is a matter of concern as Enterococci are as such intrinsically resistant to many antibiotics as well as low level aminoglycosides⁽¹⁵⁾.

Routinely a cell wall active agent like Penicillin or Vancomycin in combination with high level Aminoglycoside is recommended for treatment of the infections caused by Enterococci as this combination has synergistic effects. If there is high level aminoglycoside resistance or resistance to Penicillin- cell wall active agent, then this combination will not work, so other options should be explored. The combination would not work even if there is resistance to Vancomycin⁽¹⁵⁾. Resistance to Vancomycin is another major issue. As it is the drug of choice for most Enterococcal infections in combination with above mentioned drugs as previously widely used drug that is Erythromycin- a widely used Macrolide drug has developed high resistance towards the organism. In present study Erythromycin and fluoroquinolones have shown around 90 % resistance which shows contrast results with Alm El-Din *et al*⁽¹⁶⁾ that is 45- 50 % sensitivity of these drugs. Vancomycin resistant Enterococci accounted for 11 % which shows almost similar results with Alm El-Din *et al*⁽¹⁶⁾. Moreover, Vancomycin resistant isolates have shown multi drug resistance many a times. So treatment of these isolates is difficult as only few options are available. Linezolid, Daptomycin, Quinpristin, Teicoplanin can be given in multi drug resistant VRE isolates. There are also few limitations like Quinpristin is intrinsically resistant in *Enterococcus faecalis*, Daptomycin cannot be given in isolates from respiratory tract infections etc⁽¹⁰⁾. So the good drug options remain Teicoplanin- another glycopeptide or Linezolid-lipopeptide. Today even resistance to this group of drugs has also been noted. In present study also

sensitivity of Teicoplanin and Linezolid is 89 % and 78 % respectively which shows few isolates resistant to these drugs. Resistance to Ampicillin in present study was 56 % which shows similar results with Mohanty *et al*⁽¹⁷⁾.

One more important feature is rapid diagnosis of VRE, HLAR and multi drug resistant Enterococci which cannot be done with the help of gold standard method- Blood culture as it takes minimum of 48- 72 hours for results including incubation period, time for identification and antibiotic susceptibility. Molecular diagnostic methods which yield results within few hours are therefore mandatory for prevention of rising trend of the infection.

Conclusion & Limitations

The study shows upsurge of Vancomycin resistant Enterococci (11 %) and High level Aminoglycoside resistance (78 %) in Enterococcal neonatal septicemia (16 %), considered as only colonizers previously. With this increase in mass spread, increasing resistance to other glycopeptides, macrolides and lipopeptides leads to development of multi drug resistant Enterococci. It is a burning issue as after these drugs, no other effective treatment is available. So judicious use of Vancomycin and other higher antibiotics like Linezolid, Daptomycin, Teicoplanin should be done. Further, VRE can be transmitted from one neonate to another, so Active surveillance culture programs with more such studies should be done to identify colonizers, understand resistance pattern of the organisms and to curtail morbidity and mortality in neonates. In addition to that, periodic training and surveillance are needed to control and increase awareness amongst the health care workers about the rising multi drug resistant infectious incidence of Enterococci.

The sample size of the study was too small to comment well. We even could not differentiate Enterococci up to the species level due to constrain resources like unavailability of Arabinose test, gelatin liquefaction test, xylose

test and because of that could not comment on Quinpristin sensitivity of the isolates. We even could not identify Enterococci by means of molecular method and could not comment on presence of resistant genes present in the species.

References

1. Yilema A, Moges F, Tadele S, Endris M, Kassu A, Abebe W. Isolation of enterococci, their antimicrobial susceptibility patterns and associated factors among patients attending at the University of Gondar Teaching Hospital. 2017;1–8.
2. Furtado I, Cristhina P, Xavier N, Venhofen L, Tavares M, Alves F. Enterococcus faecium AND Enterococcus faecalis IN Blood of Newborns with Suspected Nosocomial Infection. 2014;56(1):77–80.
3. SFernandes SC, Dhanashree B. Drug resistance & virulence determinants in clinical isolates of Enterococcus species. 2013;(May):981–5.
4. Choudhry O, Gathwala G, Singh J. Vancomycin Resistant Enterococci in Neonatal ICU- A Rising Menace. 2010;1446–7.
5. Article R. International Journal of Pharma and Bio Sciences ISSN. 2012;3(3):781–6.
6. Murray BE. Vancomycin resistant enterococci. Am J Med. 1997;101:284–93.
7. Rice LB. Emergence of vancomycin resistant enterococci. Available from: <http://www.cdc.gov/ncidod/eid/vol7no2/rice.htm> [cited in 2001] [updated in 2005]
8. Gb S, Nagarathnamma T, Tr H, Karthik R. Neonatal Septicaemia Caused by Vancomycin Resistant Enterococcus Faecium -A Case Report. 2014;10–1.
9. Fisher K, Phillips C. The ecology , epidemiology and virulence of Enterococcus. 2017;(2009):1749–57.
10. The Clinical and Laboratory Standards Institute. M100S Performance Standards for Antimicrobial Susceptibility Testing. 2016.
11. Aher CS. Original Research Article Vancomycin resistant Enterococci: an emerging threat. 2014;3(2):14–9.
12. Spironello RA, Botura-amado C, Karine T, Dalva M, Carvalho DB, Martins DA. A Report on Antibiotic Management of Neonatal Sepsis Caused by Enterobacter spp. 2015;14(November):2131–4.
13. Herrmann DMML, Amaral LMB, Almeida SC. Fatores de risco para o desenvolvimento de sepse neonatal tardia em uma unidade de terapia intensiva. Pediatria(São Paulo). 2008;30:228-36.
14. Mahafzah AM, Abu-Khader IB, Bakri FG (2008). Characterization of Enterococci Causing Nosocomial Infections at the Jordan Univ. hospital over a five year period. J Med. J. 2008, 42:1-9.
15. Adhikari L. High-level Aminoglycoside Resistance and Reduced Susceptibility to Vancomycin in Nosocomial Enterococci *Journal of Global Infectious Diseases*. 2010;2(3):231-235.
16. El-din RAA, El-mahdy HS. Molecular characterization of enterococcus strains isolated from cases of neonatal sepsis in neonatal intensive care unit. 2012;6 (44):7206–11.
17. Mohanty S, Jose S, Singhal R, Sood S, Dhawan B, Das BK (2005). Species prevalence and antimicrobial susceptibility of enterococci isolated in a tertiary care hospital of North India. Southeast Asian J. Trop. Med. Public Health 36:962-965.