

Bacteriological Profiles And Antibiotic Susceptibility In Neonatal Sepsis: Development Of An Antibiogram In A Secondary Care Hospital Of South India

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ABSTRACT

Background: Neonatal Sepsis is a major cause of neonatal morbidity and mortality worldwide and particularly in low and middle income countries (LMIC's) such as India. The prevalence of bacterial isolates and their sensitivity to antimicrobial agents change from one region to another and with time and hence it is important to know the epidemiology of bacterial isolates and their antibiotic sensitivity in hospital environment to direct the empirical antimicrobial therapy.

Aim: The present study aimed to study the prevalence and to determine the bacteriological profile and antibiotic susceptibility patterns of all positive cultures in the site of neonatal sepsis with a view of forming a localised antibiogram.

Methods: Retrospective observational study was done from June 2021-June 2024 at NICU of a secondary care hospital. Every positive results of cultures in neonates were analyzed. Neonates had blood, urine, sputum, Umbilical venous catheter (UVC), Umbilical arterial catheter (UAC), Peripherally inserted central catheter (PICC), Central line blood, Cerebrospinal Fluid (CSF), and eye swabs. The bacterial isolates and culture sensitivity pattern were retrieved from the hospital's information system (HIS) and the data was recorded. The data obtained were then documented, coded and analysed on SPSS Software.

Results: 36 microorganisms were isolated from 429 positive cultures. The gram-negative isolates represent approximately 80.4% of the total bacterial isolates. Klebsiella pneumoniae (30.8%) was the most frequent pathogen in addition to Escherichia coli (25.4%). In gram positive isolates, Staphylococcus aureus (3.7%) was a common pathogen. The resistance rate of isolates was high (100%) in most groups for Ceftriaxone-cefotaxime, cefuroxime and cefipime.

Conclusion: This study demonstrated that there was high resistance of both gram-positive and gram-negative isolates to commonly used first line drugs. The rate of antibiotic resistance continues to rise in neonatal sepsis and there is a need for surveillance of microorganisms and antibiotic sensitivity pattern to take place periodically..

Keywords: Neonatal sepsis, Bacterial isolates, antibiotic sensitivity, Klebsiella, Neonatal Intensive Care Unit (NICU).

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INTRODUCTION

Sepsis in neonates remains a major cause of morbidity and mortality globally particularly in LMICs. Neonatal infections are thought to contribute to about 1/3 of the total number of newborn deaths. This burden in a developing country such as India is greater than that of more developed countries, partly because of the presence of a high proportion of premature babies, babies with low birth weights, and also for the lack of proper infection control measure and delayed diagnosis and treatment.

Sepsis in newborns (or neonatal sepsis) is a clinical syndrome associated with clinical signs of infection in the

absence or presence of bacteraemia in the first 28 days of life. It is usually classified into two categories depending on the onset of the disease as early onset and late onset sepsis. Each are associated with specific risk factors and microbiological aetiologies. Early onset sepsis is often associated with vertical transmission from mother and is often associated with community or health service onset sepsis.

Neonatal sepsis bacteriological profiles differs greatly between geographical areas and medical environments. Commonly involved are gram-positive bacteria such as Staphylococcus aureus and coagulase-negative staphylococci, and gram-negative bacteria such as

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Klebsiella pneumoniae, *Escherichia coli*, and *Pseudomonas aeruginosa*. Local epidemiological data is also very useful as there has been much variation in the incidence of specific infections and pattern of antibiotic sensitivity between secondary and tertiary care hospitals.

Global Burden of Disease (GBD) data show that the burden of neonatal sepsis is steadily changing: there are an estimated 1.3 – 3.9 million cases of neonatal sepsis every year before 28 days of age.^{1,2}

Neonatal Sepsis is a significant cause of morbidity and mortality among neonates globally and is the third most common cause of neonatal mortality, especially in developing countries like India.³ The microorganisms causing neonatal sepsis and the antibiotic susceptibility have changed over time, and depending on the geographical region.⁴ Emergence of multidrug resistance has become a global concern and a significant challenge in the successful management of neonatal sepsis.⁵ The epidemiology of bacterial isolates and their antibiotic susceptibility pattern in hospital settings is essential for prompt empirical therapy in order to reduce morbidity and mortality.

Antibiogram is a useful tool to aid in informing antibiotic prescribing decisions and antimicrobial stewardship as it presents the susceptibility patterns of common organisms over a chosen period of time. Although it is important, some of the primary limitations in the antibiograms of secondary care institutions exist in the dearth of such data from institutions from the rural and part of the urban Indian context, which has a widely different resource availability and load of patients compared with tertiary care institutions. The present study will aim at finding the bacteriological profile of newborn sepsis to determine the antibiotic susceptibility patterns of isolated pathogens in a secondary care hospital in South India. This study aims to generate a local antibiogram that will aid physicians in providing the most appropriate initial treatment (empirical antibiotic therapy) for patients in this unit, improve clinical outcomes, and support antimicrobial stewardship initiatives in the neonatal setting.

METHODOLOGY:

Study Design and Setting: Retrospective study setting within a hospital. The study was conducted in an NICU of secondary care hospital, South India: Rural Development Trust (RDT) Bathalapalli.

Study Period: This was a study from June 2021 to June 2024.

Ethics Committee Approval: The Institutional Ethics Committee (IEC) of RDT Hospital has approved it (Approval No: RDTH/BTP/ETHICS/2024/24). Confidentiality of patients was maintained and the research was conducted ethically.

Inclusion: Any cultures that were sent from the NICU are included who were between the ages of 0-28 days.

Sample Size Calculation: Sample size was calculated by using the formula:

$$n = \frac{Z_{1-\alpha/2}^2 * resistance * (1 - resistance)}{(d)^2 * prevalence (p)}$$

where,

Z= 1.96 {standard value for 95% confidence interval}

p= 0.20 {prevalence of neonatal sepsis based on prior article where *klebsiella* was common and with high resistance rate 0.90}

d² = 0.10 {desired precision level}

Although the sample size was calculated to be 173 patients, the study managed to analyse 429 culture-positive patients, which provided a more detailed perspective on the local antibiogram.

Data Source: The data was retrieved from Hospital Information System (HIS). Pre-defined inclusion and exclusion criteria were used in the data collection process.

- Demographic information for patients (e.g., age of admission)
- Specimen Type and Test name
- Blood, urine, CSF fluid, ET Aspirate (Endotracheal aspirate), UVC (Umbilical venous catheter), UAC (Umbilical artery catheter) and Central line blood were used to obtain bacterial strains for culture and susceptibility tests from the NICU.
- The collected specimens were processed and analysed in the microbiology lab in the hospital and microorganisms were isolated and identified.
- Culture sensitivity reports.
- To avoid duplications, the samples were called "Repeated Samples".

The identified bacterial isolates were also tested for sensitivity to each of these antimicrobial agents, by using the Kirby-Bauer (KB) disc diffusion method, and their susceptibility or resistance were documented in the microbiology lab and in the Hospital's Information system (HIS) as susceptible, intermediate, or resistant.⁶

Data were collected and compiled on Microsoft Excel through the statistical analysis. IBM SPSS version 27 was used to analyse the statistical data. Simple frequency and proportion was used to estimate the prevalence of sepsis, gram negative and positive of the bacteria and susceptibility was also described. For the test of the difference in susceptibility of the bacteria chi-square test was used. Epidemiological estimates of sensitivity/resistance to other antibiotics also were tested.

RESULTS:

The number of culture-positive patients included in the study was 429 (prevalence of positive neonatal sepsis). The most frequent source of positive cultures was blood (37.3%), followed by urine (32.4%), and eye swab (14%), UVC (5.6%), ET ASPIRATE (5.4%), UAC (4.9%), CSF Culture (0.5%).

Table-01: Table showing the Prevalence of positive neonatal sepsis in microbiology profile

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Culture type	Number	Total Percentage (%)
Blood culture	160	37.3%
Urine culture	139	32.4%
Eye swab culture	60	14%
Umbilical Venous catheter (UVC)	24	5.6%
Endotracheal Aspirate (ET ASPIRATE)	23	5.4%
Umbilical arterial catheter (UAC)	21	4.9%
Cerebrospinal fluid (CSF) culture	2	0.5%
Peripherally inserted central catheter (PICC)	0	0%
Central line blood culture	0	0%
Total	429	100%

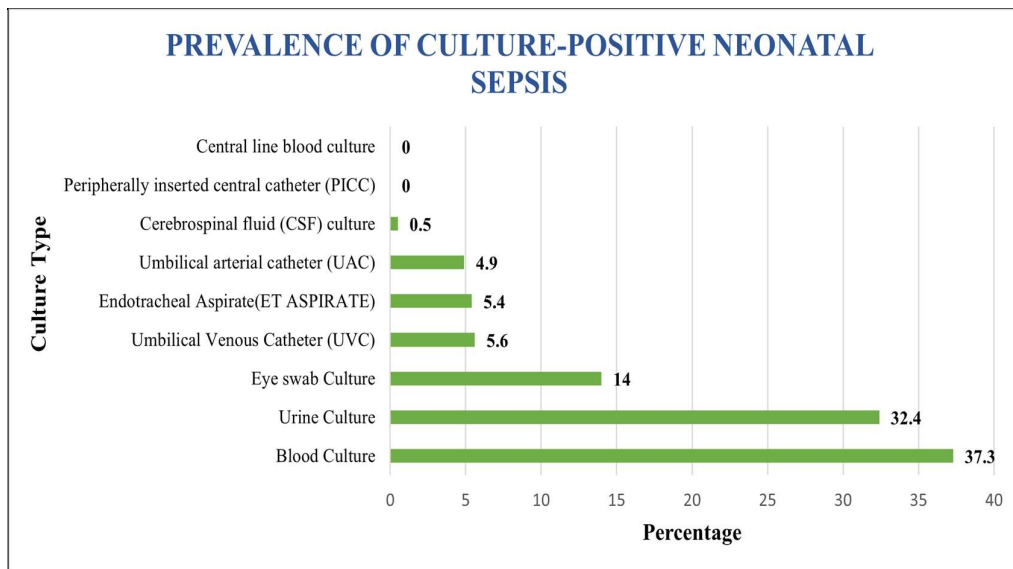


Figure-1: Prevalence of Culture-positive neonatal sepsis

Bacteriological profile of Neonatal Profiles: 36 microorganisms were isolated from a total number of 429 positive culture values. About 80.4% of the total bacterial isolates were Gram-negative organisms. The most frequent cause for Neonatal sepsis was Klebsiella pneumonia (30.8%), followed by Escherichia coli (25.4%). The other gram-negative bacteria were isolated were Acinetobacter baumannii (7.2%), Serratia marcescens (5.6 %),

Pseudomonas aeruginosa (1.9 %), and Enterobacter species (0.2 %).

Approximately 19.58% of the total number of bacterial isolates were gram-positives. Staphylococcus aureus was found as a common pathogen in Gram positive positive isolates (3.7% of isolates). Enterococcus Species (3%), Staphylococcus haemolyticus (3%), Staphylococcus epidermidis (2.1%) and Staphylococcus hominis (1.9%) were the other Gram positive isolates.

Table-02: Bacteriological profile of neonatal sepsis

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Bacterial isolates	Number (n)	(%)
Gram-negative isolates		
Klebsiella pneumoniae	132	30.8
Escherichia coli	109	25.4
Acinetobacter baumannii	31	7.2
Serratia marcescens	24	5.6
Pseudomonas aeruginosa	8	1.9
Enterobacter species	1	0.2
Gram-positive isolates		
Staphylococcus aureus	16	3.7
Enterococcus Species	13	3
Staphylococcus haemolyticus	13	3
Staphylococcus epidermidis	9	2.1
Staphylococcus hominis	8	1.9

Among Gram-negative isolates

Colistin (100%), amikacin (77.5%), meropenem (71.8%), and gentamicin (65.6%) were the most effective against Klebsiella pneumoniae. Resistances were, in contrast, very high in cephalosporins belonging to the third and fourth generation (ceftriaxone, cefotaxime, cefuroxime and cefepime). Escherichia coli isolates showed good susceptibility to colistin (97.2%), meropenem (86.1%) and amikacin (86.1%) and high levels of resistance to

cefuroxime and amoxicillin-clavulanate. The susceptibility of each group of Acinetobacter species showed complete susceptibility to colistin (100%), partial susceptibility for piperacillin-tazobactam (20.7%) and amikacin (16.7%). There were high levels of resistance against ceftriaxone, cefotaxime, cefuroxime, and amoxicillin-clavulanate.

Table-03: Antibiotic susceptibility pattern of the Gram-negative bacterial isolates

Antibiotics	Sensitivity pattern n (%)					
	Klebsiella pneumoniae	Enterobacter species	Escherichia Coli	Acinetobacter Baumannii	Pseudomonas aeruginosa	Serratia marcescens
Amikacin	100 (77.5%)	1 (100%)	93 (86.1%)	5 (16.7%)	8 (100%)	24 (100%)

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Amoxicillin/Clavulanate	24(19%)	0 (0.0%)	19 (17.6%)	0(0.0%)	1 (100%)	1 (4.5%)
Ceftazidime	32(25.4%)	0 (0.0%)	30 (27.8%)	1(3.4%)	1 (100%)	8 (33.3%)
Ceftriaxone-Cefotaxime	0 (0.0%)	1 (100%)	33 (30.8%)	0(0.0%)	0 (0.0%)	0 (0.0%)
Cefuroxime	23 (18.1%)	0 (0.0%)	16 (14.8%)	0(0.0%)	0 (0.0%)	1 (4.2%)
Colistin	128 (100%)	0 (0.0%)	105 (97.2%)	30(100%)	6 (100%)	0 (100%)
Ciprofloxacin	81 (63.7%)	3 (60%)	60 (58.8%)	5 (17.8%)	6 (85.7%)	10 (41.6%)
Cefepime	35 (29.6%)	1 (100%)	30 (29.1%)	2 (6.8%)	6 (75%)	6 (31.5%)
Meropenem	94 (71.8%)	1 (100%)	93 (86.1%)	4 (13.8%)	8 (100%)	24 (100%)
Piperacillin/Tazobactam	81(62.3%)	3 (50.5%)	79 (75.2%)	6(20.7%)	8 (100%)	21 (87.5%)
Gentamicin	86 (65.6%)	1 (100%)	74 (69.2%)	4(12.9%)	7 (87.5%)	10 (41.7%)

Among Gram-positive organisms

Staphylococcus aureus, Enterococcus species, and Staphylococcus haemolyticus were the most frequently isolated Gram-positive organisms associated with neonatal sepsis in the present study. Staphylococcus aureus demonstrated complete susceptibility to doxycycline,

linezolid, and vancomycin (100% each), while exhibiting high resistance to amikacin, piperacillin–tazobactam, ceftriaxone/cefotaxime, and cefepime. Enterococcus species, the second most common Gram-positive isolate,

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showed 100% susceptibility to vancomycin, with limited susceptibility to ciprofloxacin (28.5%).

Table-04: Antibiotic susceptibility pattern of the Gram-positive bacterial isolates

Antibiotics	Sensitivity Pattern n (%)				
	Enterococcus Species	Staphylococcus aureus	Staphylococcus epidermidis	Staphylococcus haemolyticus	Staphylococcus Hominis
Amikacin	2 (66.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Azithromycin/Erythromycin	0 (0.0%)	3 (20.0%)	2 (22.2%)	1 (8.3%)	2 (25.0%)
Piperacillin/Tazobactam	1 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Ceftriaxone/Cefotaxime	1 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Clindamycin	0 (0.0%)	10 (66.7%)	3 (33.3%)	8 (61.5%)	3 (37.5%)
Chloramphenicol	2 (66.7%)	14 (93.3%)	9 (100%)	13 (100%)	7 (87.5%)

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Cloxacillin	0 (0.0%)	5 (31.3%)	1 (11.1%)	1 (9.1%)	4 (50%)
Ciprofloxacin	4 (28.5%)	2 (12.5%)	5 (55.5%)	3 (23.0%)	3 (37.5%)
Cefepime	1 (50%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Doxycycline	6 (60.0%)	15 (100%)	9 (100%)	11 (84.6%)	8 (100%)
Gentamicin	1 (33.3%)	13 (81.3%)	3 (33.3%)	5 (41.7%)	2 (25.0%)
Linezolid	2 (100%)	15 (100%)	9 (100%)	13 (100%)	8 (100%)
Vancomycin	11 (100%)	16 (100%)	9 (100%)	13 (100%)	8 (100%)

Antibiogram

Major Heading: Gram (-), Gram (+), # Number of isolates, Penicillin's, Aminoglycosides, Cephalosporins, Others.

Description: The first column lists the organisms that were included, separated by Gram-negative and Gram-positive results. The second column shows the number of isolates

were included in the antibiogram. The remaining columns of the antibiogram are the antibiotics that were tested and the organisms susceptibilities. Yellow highlighting in any row indicates that an insufficient number of isolates were included in the antibiogram (<30).

Gram (-) Highlighted rows include less than 30 isolates: Interpret these results with caution	# Number of Isolates	Penicillin's			Aminoglycosides		Cephalosporins			Others		
		Amoxicillin/Clavulanate	Piperacillin/Tazobactam	Amikacin	Gentamicin	Ceftazidime	Ceftriaxone-cefotaxime	Cefuroxime	Colistin	Meropenem		
Klebsiella pneumoniae	132	19	62.3	77.5	65.6	25.4	0	18.1	100	71.8		
Escherichia Coli	109	17.6	75.2	86.1	69.2	27.8	30.8	14.8	97.2	86.1		
Acinetobacter Baumannii	31	0	20.7	16.7	12.9	3.4	0	0	100	13.8		
Serratia marcescens	24	4.5	87.5	100	41.7	33.3	0	4.2	0	100		
Pseudomonas aeruginosa	8	100	100	100	87.5	100	0	0	100	100		
Enterobacter species	1	0	50.5	100	100	0	100	0	0	100		
Gram (+) Highlighted rows include less than 30 isolates: Interpret these results with caution	# Number of Isolates	Penicillin's			Aminoglycosides		Others					
		Cloxacillin	Piperacillin/Tazobactam	Amikacin	Gentamicin	Ceftriaxone/Cefotaxime	Doxycycline	Chloramphenicol	Clindamycin	Linezolid	Azithromycin/Erythromycin	Vancomycin
Staphylococcus aureus	16	31.3	0	0	81.3	0	100	93.3	66.7	100	20	100
Enterococcus species	13	0	33.3	66.7	33.3	50	60	66.7	0	100	0	100
Staphylococcus haemolyticus	13	9.1	0	0	41.7	0	84.6	100	61.5	100	8.3	100
Staphylococcus epidermidis	9	11.1	0	0	33.3	0	100	100	33.3	100	22.2	100
Staphylococcus Hominis	8	50	0	0	25	0	100	87.5	37.5	100	25	100

Figure-2: The above antibiogram uses three years of culture data for these organisms

DISCUSSION:

Neonatal sepsis is recognized to be one of the leading causes of mortality and morbidity in developing countries like India. The infectious organism profile of neonatal septic cases along with antibiotic susceptibility can be of great help in choosing one's empirical antimicrobial treatment for neonatal sepsis.^{4,6,7,8} The common microorganisms associated with neonatal sepsis are of change depending on time of infection and geographical area.⁹

The rate of blood culture positivity was 37.3% (160 / 429) in this study. In the previous studies conducted in Indians, the proportion of culture positivity was found to vary from 16% to 54% depending on the place and time of the study.^{4,10,11,12} Out of 5200 culture collected from NICU, 429 became positive. This is particularly true for 37.3% (160/429) who had positive blood cultures. Many variations have occurred in the number of positive culture results from blood over the years. Murty et al. reported a higher isolation rate at 52.6% and Rajendra Prasad et al. reported the isolation at 47.5%.^{13,14} On the other side, prevalence of positive culture proved to be 20.5% and 15.3% as per studies conducted by Tasneem Siddiqui et al. and Bishma pokhrel et al. respectively.

The results of this study indicated that 32.4% (139/429) of the urine samples were positive. Other studies, however, have yielded different figures: Abdulrahman et al found lower level of positive urine culture (12.6%).¹⁵

Of the 429 positive cultures, 5.6% were positive UVCs. The studies by Ming-Yan Hei et al. showed higher number of UVC positive cultures which is reported as 9.5%.¹⁶

In this study 0.5 % of people were found to have bacterial meningitis. In contrast, all three previous studies report the prevalence of culture-confirmed bacterial meningitis in

suspected neonates to be 2.9%, 4.4%, and 5.4% and the most common pathogen to be Gram-negative.¹⁷ The same trend with regard to bacterial isolates has been previously noted in several other investigations.

Gram-negative organisms were responsible for 63.8% of all positive cultures. Shubhangi Kedar et al indicated 75.5% of Gram negative organisms and Muley et al indicated 70.8% of Gram negative organisms was the leading organism Klebsiella pneumonia.^{8,18} Developed on the basis of NNPD Report 2002-2003 and research done by Roya et al.¹⁹

The incidence of Klebsiella species was reported to be 53.6% (ranging from 50.7% to 56.5%) in India, 33% (ranging from 3.0% to 63.0%) in Nepal, and 60% in Sri Lanka.²⁰ Other gram-negative organisms isolated included Escherichia coli, Acinetobacter baumannii, and Serratia marcescens. E.coli is frequently found (Moncef et al.).²¹

Staphylococcus aureus, the most common gram-positive isolate, was found in this study. Other gram-positive organisms isolated were Enterococcus faecalis, Enterococcus species and Staphylococcus haemolyticus which are similar to the study conducted by Deepeshwara Nepal et al.²² and Karthikeyan et al.²³

The results of this study indicate a multi-drug resistance to the routinely used antimicrobial agents such as Ceftriaxone and Cefotaxime in most of the isolates. KP had the highest susceptibility to colistin (100%), amikacin (77.5%), meropenem (71.8%) and Gentamicin (65.6%) when compared to other Gram-negative pathogens. Escherichia Coli had 97.2% sensitivity to Colistin, 86.1% sensitivity to amikacin and meropenem.

Staphylococcus aureus, Enterococcus Species, and Staphylococcus haemolyticus were seen to be 100% sensitive to linezolid and vancomycin. Additionally, Staphylococcus aureus was 100% sensitive to doxycycline, 93.3% sensitive to Chloramphenicol and 81.3% to

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gentamicin. As in other studies, the gram-positive isolates in this study were most responsive to linezolid and vancomycin.^{5,24,25}

Based on the results, this research recommends that empirical antibiotic therapy for suspected sepsis in the newborn should include gram-negative as well as gram-positive bacteria and specifically include *Klebsiella pneumoniae* and *Staphylococcus aureus*. There is an increasing trend of antibiotic resistance to first-line antibiotics and even to third-generation cephalosporins. Rapid changes in sensitivity patterns have made ongoing surveillance of antibiotic sensitivity with a view to appropriate empirical treatment necessary before the results of cultures are received.

CONCLUSION:

This study provides valuable insights into the bacteriological features and the resistance to antimicrobial agents of neonatal sepsis. The findings suggest that neonatal sepsis is still a major cause of morbidity and mortality and the predominant bacteria seen were Gram-negative bacteria (Mostly *Klebsiella pneumoniae* and *Escherichia coli* were recognized as predominant pathogens).

The study shows resistance shown by both Gram-negative and Gram-positive bacteria to commonly prescribed first-line antibiotics, particularly third- and fourth-generation cephalosporins, like ceftriaxone, cefotaxime, cefuroxime, and cefepime. The rising trend of antimicrobial resistance now poses great danger to the effective treatment of neonatal sepsis and underscores the shortcomings of empirical treatment suggested by treatment guidelines.

Some antibiotics have a high level of sensitivity, such as colistin, meropenem and amikacin for Gram-negative and vancomycin and linezolid for Gram-positive bacteria. However, these are more sophisticated antibiotics and the problem of subsequent resistance and possible toxicity developing coupled with the cost are concerns.

In this study, establishment of local antibiograms plays a vital role in selecting a good empirical antibiotic treatment. There is support provided for the initiatives undertaken to optimise antibiotics usage and decrease antibiotic resistant bacteria (ARB) which are related to antibiotic stewardship. In summary, antimicrobial resistance trends of bacterial pathogens will need to be monitored on an ongoing basis. Continuous hospital antibiograms and practices to enhance outcomes for patients with neonatal sepsis.

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