Original Article

Bacteriological Profile and their Susceptibility Pattern in Neonatal Intensive Care Unit at Tertiary Care Hospital in Wah

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[1,4,5] *Experimentation/Study conduction*
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[2,6] *Critical Review*
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Article Processing
Received: 03/01/2020
Accepted: 21/08/2020

Cite this Article: *Mushtaq, S., Ashraf, S., Ghazal, L., Zahid, R., Hussain, B., Jamila. Bacteriological Profile and their Susceptibility Pattern in Neonatal Intensive Care Unit at Tertiary Care Hospital in Wah. Journal of Rawalpindi Medical College. 30 Sep. 2020; 24(3): 219-224.*
DOI: https://doi.org/10.37939/jrmc.v24i3.1310

Conflict of Interest: *Nil*
Funding Source: *Nil*

Access Online:

Abstract

Introduction: Neonatal sepsis is a clinical syndrome characterized by multiple symptoms and signs of infection during the first month of life. The objective of this study is to determine the frequency of commonly isolated bacteria from patients of neonatal sepsis and their susceptibility patterns in POF hospital at Wah.

Methods: This cross-sectional study was carried out in POF Hospital Neonatal intensive care unit and Microbiology laboratory from January 2018 to December 2019. The blood samples of patients suspected of neonatal sepsis were processed as per standard methodology.

Results: Out of ninety blood samples, fifty-one (56.7%) yielded the growth of Gram-negative rods and thirty-nine (43.3%) yielded Gram-positive cocci. Among Gram-positive bacteria, coagulase-negative staphylococci were the most common pathogen isolated from 53.8% cases followed by methicillin-resistant *Staphylococcus aureus* (15.3%). Among Gram-negative bacteria, *Klebsiella pneumoniae* (54.90%) was the most frequently identified bacteria followed by *Serratia marcescens* (27.45%). The Gram-positive cocci were the most susceptible to linezolid (100%) followed by vancomycin (87.2%). The Gram-negative rods depict remarkable resistance to ciprofloxacin (92.2%), gentamicin (100%), and meropenem (54.9%).

Conclusions: The study concluded a predominance of Gram-negative bacteria as a causative agent of neonatal sepsis in our setup. The bacterial isolates are highly resistant to commonly prescribed oral as well as injectable antibiotics. Implementation of infection control policies is a dire need to combat the grave situation of increasing antibiotic resistance.

Keywords: Neonatal sepsis, Antibiotic susceptibility, Bacteriological profile.

Introduction

Neonatal sepsis (NNS) is an invasive bacterial infection that occurs in the first month of life and a major cause of neonatal morbidity and mortality. It is a clinical syndrome characterized by multiple and nonspecific signs of infection which include lethargy, less vigorous sucking, apnea, bradycardia, temperature instability, respiratory distress, vomiting, diarrhea, and abdominal distention. The term also encompasses bloodstream infections, meningitis, and pneumonia.[1] NNS is the third most common cause of deaths among neonates, accounting for 225,000 deaths globally every year.[1]

Neonatal sepsis is divided into two categories based on the time and peripartum pathogenesis. Sepsis which occurs in the first 72 hours of life is defined as early-onset sepsis (EOS) and that occurring beyond 72 hours is defined as late-onset sepsis (LOS). Earlier onset of infection reflects vertical transmission from mother to infant, while the third day of life or later is likely to be acquired through horizontal transmission.[2]

The bacterial isolates which caused neonatal sepsis vary from place to place and they keep on changing with time at the same place. In industrialized countries, the commonest bacterial pathogens isolated from septic neonates are Gram-positive cocci followed by Gram-negative bacilli and fungi.[3] However, the studies conducted in developing countries like India and Pakistan revealed the predominance of Gram-negative bacilli as causative agents of neonatal sepsis.[4,5] The contribution of both Gram-positive and Gram-negative bacteria to cause neonatal sepsis is equal in a study in Nigeria.[6] The study which is carried out at the same hospital as ours revealed the predominance of *Klebsiella species* followed by *Staphylococcus aureus*.[7] Similarly, the susceptibility pattern also has geographical as well as temporal variations. In Egypt, the commonest neonatal pathogen was coagulase-negative Staphylococcus species. The isolates were sensitive to vancomycin, ciprofloxacin, and amikacin and resistant to commonly used first-line antimicrobial drugs.[8] One of the studies conducted in India depicted rampant resistance to cephalosporins and susceptibility of Gram-negative bacilli against piperacillin-tazobactam whereas vancomycin and linezolid were most effective against Gram-positive isolates.[9] The data by Tehseen et al revealed that amikacin and vancomycin were the most effective antimicrobial drugs against Gram-negative and Gram-positive bacteria respectively.[7] The variable microbiological pattern and their antibiograms render antibiotic susceptibility profile in one region at a specific period inapplicable to other regions or in another period. Keeping in view, the present study was aimed to document the commonly isolated bacteria from patients of neonatal sepsis and their susceptibility patterns in POF hospital at Wah. This will be an effort to rationalize the empirical treatment by paediatricians resulting in evidence-based practice and better results in terms of early recovery, shorter duration of hospital stay, and cost-effectiveness. Moreover, this effort will contribute to safeguard the remaining therapeutic options left to the clinicians and encourage a focused, concerted effort for better patient care.

Materials and Methods

This cross-sectional study was conducted at the neonatal intensive care unit and laboratory of Pakistan Ordinance Factories Hospital from January 2018 to December 2019. The sample size was calculated by the WHO sample size calculator taking a confidence level of 95%, population size[7] of 117, and a margin of error of 5%. The sample size (n) was 90. The samples were calculated by the non-probability consecutive technique. All neonates of either gender, irrespective of risk factors, with clinical suspicion of neonatal sepsis were included in the study. Patients who were already on antibiotics were excluded. The blood culture samples were drawn from all neonates who were clinically suspected of neonatal sepsis, without any discrimination of gender, body weight, prematurity, and mode of delivery. According to institution protocol, 1-3 ml of blood was drawn from a peripheral vein and inoculated in Brian heart infusion (BHI) broth for culture. The blood samples were incubated for 24 hrs at 35 $\pm$ 2°C under aerobic conditions. Blind subcultures were done by collecting the inoculums from BHI broth and inoculating on blood agar and MacConkey agar after 24 hours, 72 hours, 5th day, and on 7th day. The subcultures were incubated at 35 $\pm$ 2°C under aerobic conditions. After overnight incubation, the agar plates were examined for growth of bacteria and their colonial morphology. For Gram-positive cocci identification, catalase test, coagulase test, growth on bile esculin agar, and salt tolerance test were employed. The Gram-negative rods were identified based on Gram staining, catalase test, oxidase test, and motility.[10] Microbact Gram-negative 24E identification kits (Oxoid, Basingstoke, UK) were used for confirmation of Gram-negative isolates.

Antimicrobial susceptibility tests were performed on the Muller–Hinton agar plates with the disk diffusion method as recommended by the clinical laboratory standards institute.[11] The bacterial suspensions of isolates equivalent to 0.5 McFarland standard turbidity were applied on Mueller-Hinton agar (Oxoid, Basingstoke, UK). Following antimicrobial disks (Oxoid, Basingstoke, UK) were evenly placed on the inoculated plates for Gram-negative bacilli: Ampicillin(10 µg), amoxicillin-clavulanate (20/10 µg), trimethoprin-sulfamethoxazole (1.25/ 23.75 µg), ceftriaxone (30 µg), cefotaxime (30 µg) ciprofloxacin (5 µg), gentamicin (10 µg), amikacin (30 µg), piperacillin-tazobactam (100/ 10 µg), doxycycline (30 µg) imipenem (10 µg) and meropenem (10 µg) and polymyxin B(300 units)

The disks of penicillin (10 units), cefoxitin (30µg), amoxicillin/clavulanic acid (20/10 µg), erythromycin (15 µg), clarithromycin (15 µg), linezolid (30 µg), ciprofloxacin (5µg), clindamycin (2µg), doxycycline (30µg) and vancomycin (30µg) were applied for Gram-positive cocci.

Concurrent quality control testing was performed with *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923.

After overnight incubation, the diameter of each zone of inhibition around the antimicrobial disk was measured. The susceptibility results were interpreted as sensitive, intermediate, and resistant according to recommendations of CLSI.[11]

The data was entered and analyzed using SPSS version 21. For qualitative variables (Gram-negative bacilli, Gram-positive cocci, gender, bacteria isolated, and their susceptibility pattern) frequencies and percentages were calculated. Mean ± SD was presented for age. Association of Gram-negative bacilli and Gram-positive cocci with early and late-onset neonatal sepsis was determined by the chi-square test. p-value $\leq$ 0.05 was considered significant.

Results

A total of ninety positive blood cultures of neonates with bacterial sepsis were analyzed. The mean age of the neonates was 6.68 days $\pm$ 7.17. In a total of ninety, forty-seven (52.2%) cases of neonatal sepsis were categorized as early onset and forty-three (47.8%) cases were categorized as late-onset.

Out of ninety, fifty-one specimens (56.7%) yielded the growth of Gram-negative rods and thirty-nine (43.3%) specimens yielded Gram-positive cocci. The gender distribution for 39 Gram-positive cocci was 25 and 14 for males and females respectively. Out of 51 Gram-negative isolates, 31 were isolated from male patients and 20 were isolated from females. This gender distribution is presented in Figure 1. There was no significant association of Gram-positive cocci and Gram-negative rods with early and late neonatal sepsis. (p=1.00)

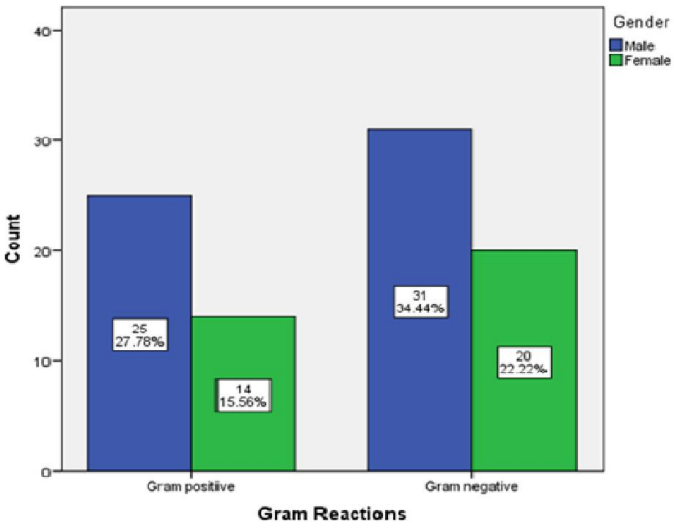

Figure 1: Distribution of Gram-positive cocci and Gram-negative bacilli isolated from the blood of neonatal sepsis in both genders (n=90)

The most frequent bacteria isolated from the blood of neonatal sepsis were *Klebsiella pneumoniae* followed by coagulase-negative staphylococci and *Serratia marcesens*. Among Gram-positive bacteria, coagulase-negative staphylococci were the most common pathogen isolated from 53.8% cases followed by methicillin-resistant *Staphylococcus aureus* (15.3%). Among Gram-negative bacteria, *Klebsiella pneumoniae* (54.90%) was the most frequently identified bacteria followed by *Serratia marcescens* (27.45%) and Acinetobacter species (9.8%). (Figures 2 and 3)

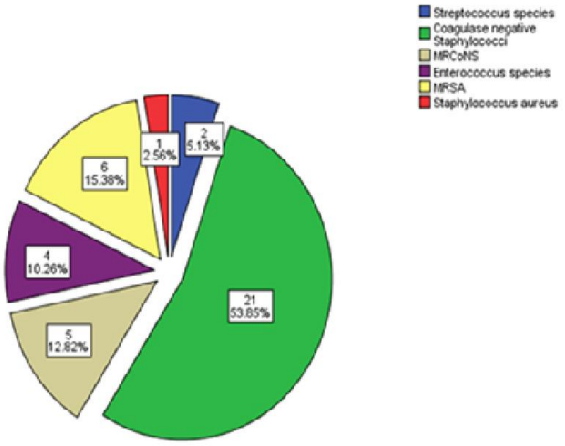

Figure 2: Gram-Positive cocci responsible for Neonatal sepsis (n= 39)

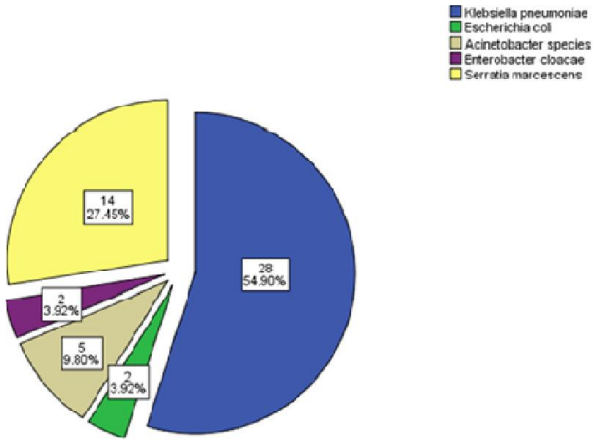

Figure 3: Gram-Negative bacilli responsible for Neonatal sepsis (n= 51)

The Gram-positive cocci were markedly resistant (76.9%) to first-line drugs including penicillin. A moderate level of sensitivity was exhibited by Gram-positive isolates to amoxicillin-clavulanate, ciprofloxacin, clindamycin, macrolides, doxycycline, and gentamicin. Amikacin and meropenem were effective in 65.7% and 61.5% of cases of neonatal sepsis caused by gram-positive pathogens. These organisms were the most susceptible to linezolid (100%) followed by vancomycin (87.2%). The exact number and percentages of the susceptibility of commonly used antimicrobials against Gram-positive cocci are represented in Table 1.

Table 1: Cross-tabulation of Gram-positive cocci against susceptibility pattern of antimicrobial drugs

	Sensitive n (%)	Resistant n (%)	Total n (%)
Penicillin	9 (23.1)	30 (76.9)	39 (100)
Ciprofloxacin	17 (43.6)	22 (56.4)	39 (100)
Erythromycin	16 (45.7)	19 (54.3)	35 (100)
Clarithromycin	17 (48.6)	18 (51.4)	35 (100)
Doxycycline	19 (48.7)	20 (51.3)	39 (100)
Gentamicin	19 (48.7)	20 (51.3)	39 (100)
Amoxicillin-clavulanate	20 (51.3)	19 (48.7)	39 (100)
Clindamycin	18 (51.4)	17 (48.6)	35 (100)
Meropenem	24 (61.5)	15 (38.5)	39 (100)
Amikacin	23 (65.7)	12 (34.3)	35 (100)
Vancomycin	34 (87.2)	5 (12.8)	39 (100)
Linezolid	39 (100)	0 (0)	39 (100)

The Gram-negative rods were 100% resistant to ampicillin and gentamicin. The commonly prescribed third-generation cephalosporins, ciprofloxacin, and amoxicillin-clavulanate were also markedly ineffective for Gram-negative isolates. These rods were moderately sensitive to amikacin, meropenem, and piperacillin-tazobactam (41.2%, 45.1%, and 41.2% respectively). Table 2 shows the susceptibility pattern of the Gram-negative rods against various antibiotics.

Table 2: Different age groups in the study population

	Sensitive n (%)	Resistant n (%)	Total n (%)
Ampicillin	0 (0.0)	51 (100)	51 (100)
Gentamicin	0 (0.0)	51 (100)	51 (100)
Cefotaxime	1 (2.0)	50 (98.0)	51 (100)
Ceftriaxone	1 (2.0)	50 (98.0)	51 (100)
Amoxicillin-clavulanate	4 (7.8)	47 (92.2)	51 (100)
Ciprofloxacin	4 (7.8)	47 (92.2)	51 (100)
Cotrimoxazole	6 (11.8)	45 (88.2)	51 (100)
Doxycycline	10 (20.0)	40 (80.0)	51 (100)
Amikacin	21 (41.2)	30 (58.8)	51 (100)
Piperacillin-tazobactam	21 (41.2)	30 (58.9)	51 (100)
Meropenem	23 (45.1)	28 (54.9)	51 (100)
Polymyxin	37 (72.5)	14 (27.5)	51 (100)

Discussion

Neonatal sepsis is a life-threatening condition associated with poor maternal health, illiteracy, lack of medical facilities, non-compliance to antenatal visits, and non-professional handling of deliveries. The knowledge of causative pathogens and their antimicrobial susceptibility is of utmost importance to reduce neonatal morbidity and mortality. The current study assessed that Gram-negative rods are responsible for the majority of cases (56.66%) of neonatal sepsis in our setup followed by Gram-positive cocci (43.34%). This is also noticed that there is a mild increase in the incidence of early onset of neonatal sepsis as compared to late-onset neonatal sepsis in our setup. This finding is congruent with the study conducted by Adatara et al[12] where they found the majority of their patients having the early onset of neonatal sepsis. The study conducted by Dalal in India also showed a similar trend.[13] Early-onset neonatal sepsis is by the vertical transmission of pathogens from the female urogenital tract to the newborn either in utero or during delivery. Other risk factors include chorioamnionitis, prematurity, and prolonged rupture of membranes. Late-onset neonatal sepsis is caused by the postnatal acquisition of the pathogens which thrive in the hospital or home, after contact from healthcare workers or caregivers. A possible explanation for the lower incidence of LONS could be improved practices and better understandings of cleanliness and the use of aseptic techniques by hospital staff.[14] The incidence of NNS, irrespective of the age of presentation, was seen more in male patients as compared to females. The literature review supports the increased propensity of male patients to sepsis which is attributed to gender-specific genes related to the immune system.[15] Our study revealed the predominance of Gram-negative bacilli as the causative agent of neonatal sepsis which is comparable to a vast number of studies conducted in Asia including Pakistan.[4,5,7] Among Gram-negative rods, *Klebsiella pneumoniae* was the most frequent bacteria followed by *Serratia marcescens* and *Acinetobacter species*. This pattern is different when compared to the previous study at the same place.[7] *Serratia* and *Acinetobacter* spp had emerged as new pathogens in our neonatal ICU. Moreover, a reduction in the susceptibility of Gram-negative rods against amikacin and amoxicillin-clavulanate has been recorded from 76.9%, 24.8%, 41.2%, and 7.8% respectively. The susceptibility against gentamicin and ampicillin has been reduced to zero percent each from 39.35% and 6.8% respectively. Only 2% of the isolates are sensitive to the commonly used third-generation cephalosporins. This trend of increased resistance is an alarming situation that emerged as a result of poor infection control measures as well as injudicious use of antibiotics. Similar susceptibility patterns have been reported in other studies from Pakistan.[16] The current situation of antimicrobial resistance in most of the clinical setups is in contrast to data published in an English surveillance programme for antimicrobial utilization and resistance (ESPAUR) report, 2018.[17] The difference emphasizes the need for antimicrobial stewardship programmes and surveillance of antimicrobial resistance in our health care facilities. Meropenem is the most commonly used carbapenem in the paediatric age group for life-threatening infections. The Gram-negative isolates resistant to meropenem were 54.9% and the Gram-positive isolates resistant to meropenem were 38.5%. These findings are congruent to data of Li et al.[18]

In our study, Gram-positive isolates were sensitive to linezolid (100%) followed by vancomycin (87.2%). Comparison with the previous study at the same place revealed a decrease in the susceptibility of Gram-positive cocci against amikacin, amoxicillin-clavulanate, and vancomycin from 74.5%, 60.8%, and 95.2% to 65.7%, 51.3%, and 87.2% respectively. Interestingly, mild increases in the sensitivity of less commonly used antibiotics like erythromycin, penicillin, and doxycycline are noted in the index study. This depicts the fact of bacteria rolling back to sensitivity against the less commonly used traditional drugs. Analysis of our study also showed the inclusion of *Enterococcus* and *Streptococcus* species in the list of bacterial pathogens of our NICU in addition to Staphylococci. The situation is similar to various other studies.[4,9,19] The Gram-positive cocci are remarkably resistant to ciprofloxacin(56.45%) and clindamycin(51.4%). The misuse or overuse of antibiotics like ciprofloxacin due to its extended antimicrobial spectrum promotes bacterial resistance and limits their efficacy. In our set up linezolid and polymyxin are the most effective drugs for Gram-positive and Gram-negative bacterial isolates of NICU, respectively. Despite their maximum sensitivity, these should not be used indiscriminately and be kept as reserve drugs. The development of resistance to these drugs may leave us with no option in life-threatening infections by multidrug-resistant organisms.

Conclusion

The study concluded that Gram-negative bacteria are the predominant causative agent of neonatal sepsis in our setup. The most frequent bacteria were *Klebsiella pneumoniae* followed by coagulase-negative staphylococci and *Serratia marcesens*. The bacterial isolates are highly resistant to penicillins, cephalosporins, and quinolones. Implementation of infection control policies and antibiotic de-escalation approach is recommended to combat the grave situation of increasing antibiotic resistance.

References

1. Suman C, Sindhu S, Ramesh A, Sally E, Mike S, Sankar M et al. Neonatal sepsis in South Asia: huge burden and spiraling antimicrobial resistance.BMJ 2019; 364. DOI: https://doi.org/10.1136/bmj.k5314
2. Guo J, Luo Y, Wu Y, Lai W, Mu X. Clinical Characteristic and Pathogen Spectrum of Neonatal Sepsis in Guangzhou City from June 2011 to June 2017. Med Sci Monit. 2019 Mar 29;25:2296-2304. DOI: 10.12659/MSM.912375
3. Mutlu M, Aslan Y, Acar FA, Kader S, Bayramoglu G, Yilmaz G. Changing trend of microbiologic profile and antibiotic susceptibility of the microorganisms isolated in the neonatal nosocomial sepsis: a 14 years analysis. J Matern-Fetal Neo M. 2019; https://doi.org/10.1080/14767058.2019.1582633
4. Joshi HA, Shah SS. A study on bacteriological profile, drug sensitivity and resistance pattern of isolated organism in neonatal septicaemia in neonatal intensive care unit. Int J Contemp Pediatr. 2017; 4 :1430-3. http://dx.doi.org/10.18203/2349-3291.ijcp20172680
5. Abbasi NB, Jabeen N, Khatoon S. Neonatal sepsis; Common bacterial isolates and their antimicrobial susceptibility patterns in neonatal Intensive Care Unit, Islamabad. Professional Med J 2017;24(10):1455-1460. DOI:10.17957/TPMJ/17.3914
6. Pius S, Bello M, Galadima GB, Ibrahim HA, Yerima ST, Ambe JP. Neonatal septicaemia, bacterial isolates, and antibiogram sensitivity in Maiduguri North-Eastern Nigeria. Niger Postgrad Med J.2016; 23:146-51. DOI:10.4103/1117-1936.190340
7. Tehseen T, Qureshi AH, Ghazal L. Bacterial Isolates of Neonatal Sepsis and their Susceptibility Pattern in POF Hospital Wah Cantt. IDJ. 2018; 27 (3):70-73, retrieved from https://www.mmidsp.com/publications
8. Shehab El-Din EMR, El-Sokkary MA, Bassiouny MR, Hassan R. Epidemiology of Neonatal Sepsis and Implicated Pathogens: A Study from Egypt. BioMed Research International. 2015, Article ID 509484, 11 pages, 2015. DOI: https://doi.org/10.1155/2015/509484.
9. Panigrahi P, Chandel DS, Hansen NI, Sharma N, Kandefer S, Parida S, et al. Neonatal sepsis in rural India: timing, microbiology and antibiotic resistance in a population-based prospective study in the community setting. J Perinatol. 2017; 37(8):911-921. DOI: 10.1038/jp.2017.67. Epub 2017 May 11. PMID: 28492525; PMCID: PMC5578903.
10. Jorgensen JH, Carroll KC, Funke G, Pfaller MA, Landry ML, Richter SS, Warnock DW (editors). Manual of Clinical Microbiology. 11thed. Washington, D.C: ASM press 2015. 613-34
11. CLSI. Performance standards for antimicrobial susceptibility testing; 28th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2018
12. Adatara P, Afaya A, Salia SM, Afaya RA, Konlan KD, Agyabeng- Fandoh E, et al. Risk Factors Associated with Neonatal Sepsis: A Case Study at a Specialist Hospital in Ghana. Sci World J.2019. DOI: https://doi.org/10.1155/2019/9369051
13. Dalal P, Gathwala G, Gupta M, Singh J. Bacteriological profile and antimicrobial sensitivity pattern in neonatal sepsis: a study from North India. Int J Res Med Sci 2017;5: 1541-5. DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20171261
14. Singh M, Gray CP. Neonatal Sepsis. [Updated 2019 Jul 15]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2019 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK531478/
15. Lakhey A, Shakya H. Role of Sepsis Screening in early diagnosis of Neonatal Sepsis. JPN [Internet]. 30 Mar.2017;7(1) : 1103-10. Available from: https://www.nepjol.info/index.php/JPN/article/view/16944
16. Hussain M, Aurakzai AA, Irshad M, Ihsan Ullah. Neonatal sepsis; frequency of various bacteria and their antibiotic sensitivity in neonatal sepsis. Professional Med J 2018; 25(11):1683-1688. DOI:10.29309/TPMJ/18.4719
17. Public Health England: English surveillance programme antimicrobial utilization and resistance (ESPAUR) report - Publications - GOV.UK. 2018
18. Li JY, Chen SQ, Yan YY, YingHu Y, Wei J, Lin ZL, et al. Identification and antimicrobial resistance of pathogens in Neonatal septicemia in China— A meta-analysis. Int J Infect Dis.2018; 71: 89-93. DOI: 10.1016/j.ijid.2018.04.794
19. Pius S, Bello M, Galadima GB, Ibrahim HA, Yerima ST, Ambe JP. Neonatal septicaemia, bacterial isolates, and antibiogram sensitivity in Maiduguri North-Eastern Nigeria. Niger Postgrad Med J. 2016; 23:146-51. DOI: 10.4103/1117-1936.190340.