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Study on Multidrug, Extensive Drug and Pan Drug Resistance in Septicemic Infants

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Abstract

Background: Septicemia is common in neonates and is one of the leading causes of morbidity and mortality in the early stages of life. With positive signs and symptoms, early-stage diagnosis and treatment of neonates are important to decrease death rates and complications. This article will provide an in-depth study to evaluate the resistance pattern including multi, extensive and pan drugs resistance in septicemic infants.
Materials and Method: A descriptive cross sectional study was carried out at Rehman Medical Institute (RMI), Hayatabad Medical Hospital (HMC) and Khyber Teaching Hospital (KTH), Peshawar, Khyber Pakhtunkhwa (KP) and 999 blood samples were collected from septicemic infants (1 day to 1 year of age) belonging to both genders. Blood was screened for the presence of pathogenic bacteria and their complete data was collected and analyzed with SPSS-20.
Results: In total 999 collected blood samples, 105 (10.5%) were found positive for septicemia. In these 105 positive cases, males were 70 (66.6%) while 35 (33.3%) were female infants. Out of 105 positive cases, 66 (62.8%) revealed growth of Gram positive while 39 (37.1%) for Gram negative bacteria. The most common Gram positive bacterial isolates were *Staphylococcus aureus* 56 (53.3%) and *Enterococcus* spp 10 (9.5%) while Gram negative were *Klebsiella pneumonia* 11 (10.5%) followed by *Escherichia coli* 10 (9.5%), *Pseudomonas aeruginosa* 7(6.7%), *Acinetobacter baumannii* 5 (4.8%), *Stenotrophomonas mettophilia* 3 (2.9%) and *Enterobacter*, *Serratia* and *Citrobacter* spp (one isolate each).
Conclusion: The *S. aureus* isolates were resistant to Penicillin and Cephalosporin while were sensitive to Glycopeptides and Oxazolidinone group. The *Enterococcus* isolates were resistant to Aminoglycoside and Macrolides group while were sensitive to Glycopeptides, Rifamycin and Oxazolidinone. The isolates of *Enterobacteriaceae* were mostly resistant to penicillins and were sensitive to colistin and Piperacillin/ Tazobactam. In the current study 63.8% isolates were MDR, 2.9% XDR and luckily no PDR isolate was found.

Keywords: *Septicemic Infants, Multi Drug Resistant, Extensive Drug Resistant, Pan Drug Resistant.*

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Introduction

The presence of bacteria/bacterial toxins, which usually cause prostration and fever, is known as septicemia and is one of the most severe bacterial infections often reported in neonates (Liew *et al* 2006). Septicemia has been found to play a major role in neonatal morbidity and mortality around the globe (Shen *et al* 2017.). Sepsis has also been reported to turn into life threatening condition, causing sepsis shock or severe sepsis in later stages. Weak immune system makes infants and children the easiest targets and lung, abdominal and urinary tract infections are the common causes of sepsis. The disease then spreads and infects other organs and is distributed to vascular clumps, hence requiring quick antimicrobial treatment (Negussie *et al.*, 2015).

A variety of Gram positive and negative bacterial species have been reported as the causative agents including *E. coli*, *Klebsiella* species, *Neisseria meningitides*, *Haemophilus influenza*, *P. aeruginosa*, *Streptococcus agalactiae*, *Streptococcus pneumonia*, *Streptococcus pyogenes*, Coagulase Negative Staphylococci (CoNS), *Enterococcus faecium* and *S. aureus* and are usually identified by blood cultures (Dagnew *et al.*, 2013). Antibiotics play a key role to control bacterial infections, but due to increased and misuse of these antibiotics, the bacterial pathogens have developed different strategies to overcome the action of these antimicrobial agents. The key

strategies developed by these pathogens include; enzymes produced by genes to degrade the drugs, changing the drugs targets inside microbial cell and efflux pumps that pumps the drugs out of the cell (Gerard, 2011). The resistance to antimicrobials can be classified into different types based on number of antibiotics to which a pathogenic microorganism is resistant (Merli *et al*., 2015). The Centre for Disease Control and Prevention (CDC) have revised the concept of antibiotic resistance to better compare, explain and provide comprehension and use the terms like Multi Drug Resistant (MDR), Extensive Drug Resistant (XDR) and Pan Drug Resistant (PDR). The MDR is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) and PDR as non-susceptibility to all agents in all antimicrobial categories (Magiorakos *et al.,* 2012).

A research showed that *S. aureus* was the most prevalent bacteria in the infected children (Beaudoin, T *et al.,* 2017). The *S. aureus* along with other bacteria were susceptible to Vancomycin and the lowest sensitivity was to Aztreonam irrespective of the blood type and sex (Rivers and Ahrens 2008). In another research, out of 1060, 393 (37.1%) were declared as MDR, 146 (13.8%) XDR and no PDR were found. The bacterial species were susceptible to Colistin and Vancomycin (Basak *et al.,* 2016). Diagnosis and treatment of the neonates with positive sign and symptoms for septicemia are important to decrease death rate and complications. Different symptoms of the disease can give best predictions of positive blood samples however with low specificity and sensitivity and isolating the causing pathogens is still the gold standard for treating septicemia (Kayange *et al.,* 2010).

Therefore, the current research project was designed to evaluate the prevalence of different pathogenic species of bacteria responsible for septicemia in infants and to determine the antimicrobial sensitivity pattern in terms of MDR, XDR and PDR in study area.

Materials and methods

Study Design

The cross sectional study was designed at the Center of Biotechnology and Microbiology, University of Peshawar. A total of 999 suspected neonatal blood samples were collected from both gender with less than one year of age in an aseptic condition at Hayatabad Medical Hospital (HMC) and Khyber Teaching Hospital (KTH), Peshawar, KhyberPukhtunkhwa, Pakistan.

Inclusion and Exclusion Criteria

Neonates with complaints of fever or very mild body temperature, shaking chills, rapid pulse, fast breathing, vomiting and diarrhea were included in the study while the neonates with previous history of either fungal or parasitic infestations or those taking antibiotics were excluded.

Isolation of Bacterial Isolates

All blood samples were processed for bacterial growth using BACTEC analyzer (BD, USA), an automated blood culture system. No bacterial growth in samples after 5 days were reported negative for blood culture while positive blood culture samples were further sub-cultured on different media; blood and MacConkey agar, and were incubated for 24h at 37ºC for the bacteria growth. After sub-culturing, Gram staining was performed on all positive blood culture samples to differentiate between Gram negative and positive isolates (Thairu et al 2014). Biochemical tests like Coagulase, Catalase, and DNase were performed for Gram Positive Cocci (GPC) while Oxidase, Urease, Triple Sugar Iron (TSI) and Citrate tests were performed in case of Gram-negative bacilli (Hsueh *et al.,* 2005).

Determination of Methicillin Resistant *Staphylococcus aureus*

The GPC were further checked for confirmation of Methicillin Resistant *Staphylococcus aureus* (MRSA) using cefoxitin disc. A disc of cefoxitin was place on the inoculated culture plate of *S. aureus* and incubated for 24h at 37ºC. The zone of inhibition of the test organism less than 22mm were interpreted MRSA.

Antimicrobial Sensitivity Pattern

The *in-vitro* antibiotic susceptibility patterns of the bacterial isolates were determined according to the Kirby-Bauer disc diffusion method (CLSI, 2006). A lawn of pure culture was prepared on sterile Muller Hinton Agar (MHA) plates and selected antibiotic discs (**Table 1**) were placed on it. Afterwards, the plates were incubated for 24h at 37°C, zone of inhibition (in millimeters) were measured and susceptibility (sensitive, intermediate, or resistance) of each drug was determined after incubation. The results obtained in this way were inferred from the reference provided by Clinical and Laboratory Standard Institute 2017 (CLSI-2017).

Results

Prevalence of Bacterial Isolates

The current study screened 999 neonatal blood samples collected from HMC and KTH hospitals of Peshawar. Of the isolates, 105 (10.5%) blood samples yielded bacterial growth. Among these 105 neonates, 70(66.6%) were male patients while the remaining 35(33.3%) were female (**Fig. 1)**. Out of these 105 isolates, 66 (62.8%) were Gram positive while 39(37.1%) were Gram negative bacteria. Among Gram positive bacterial isolates, *S. aureus* was the most prevalent 56 (84.4%) followed by *Enterococcus* spp 10 (15.6%). In case of Gram negative, *Klebsiella pneumonia* was the most prevalent isolate 11(28.2%) followed by *E. coli* with 10(25.6%), *P. aeruginosa* 7(17.9%), *Acinetobacter baumannii* 5(12.8%), *Stenotrophomonas mettophilia* 3(7.6%) while one isolate was found positive for each of *Citrobacter*, *Enterobacter* and *Serratia* **(Table 1)**. All the isolates were then stored at -4ºC for further analysis.

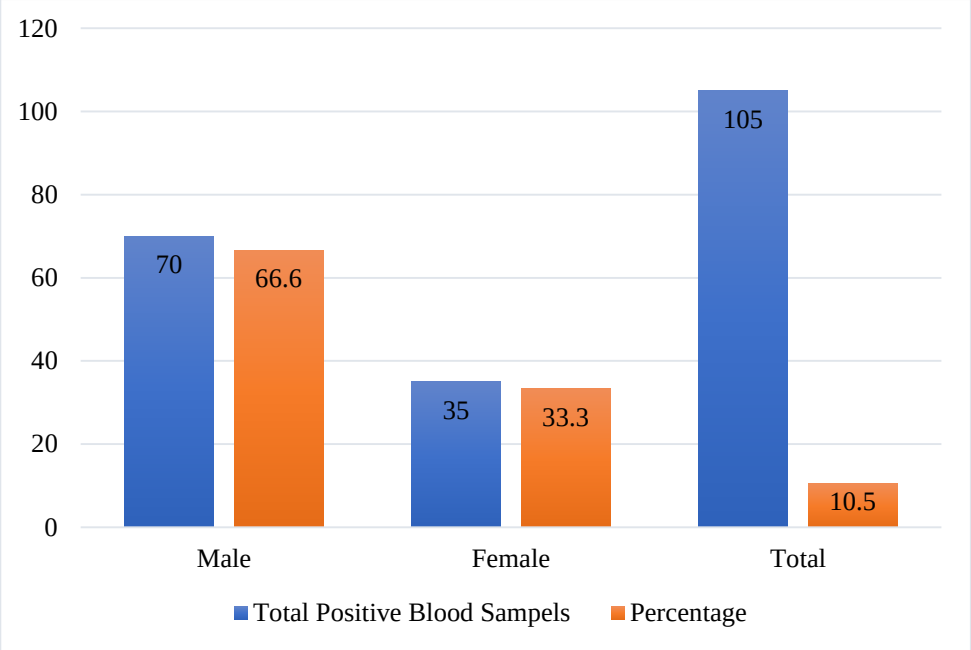

Figure 1: Prevalence and gender wise distribution of culture positive isolates (n=999).

Table 1: Frequency distribution of bacteria isolated from blood samples of neonates (n=105)

S.No	Bacterial Isolate	Frequency	Percentage
	Gram Positive bacterial isolate		
1	*Methicillin Sensitive Staphylococcus aureus* (MSSA)	11	16.6
2	*Methicillin Resistant Staphylococcus aureus* (MRSA)	45	68.1
3	*Enterococcus spp*	10	15.1
	Gram Negative bacterial isolate		
1	*Klebsiella pneumonia*	11	28.2
2	*Escherichia coli*	10	25.6
3	*Pseudomonas aeruginosa*	7	17.9
4	*Acinetobacter baumannii*	5	12.8
5	*Stenotrophomonas mettophilia*	3	7.6
6	*Enterobacter spp*	1	2.5
7	*Serratia spp*	1	2.5
8	*Citrobacter spp*	1	2.5

Identification of the Bacterial Isolates

Gram staining (microscopic examination) of the bacterial isolates were performed to differentiate between Gram positive and negative bacteria. Gram positive bacteria give purple while Gram negative give pink color after staining. Based on the results of Gram staining, 66 (62.8%) were identified as Gram positive while 39 (37.1%) as Gram negative, out of the total 105 culture positive blood samples. The Gram positive cocci with positive results for catalase, coagulase and DNase were further checked against Cefoxitin for confirmation of Methicillin Resistant *Staphylococcus aureus* (MRSA). The GNR exhibiting negative results for citrate, urease and oxidase activity were preceded for TSI to observe either yellow slope (acidic reaction) or yellow butt (acidic reaction) for the presence of *E. coli*. For Gram Negative rods identification like *Pseudomonas* species, isolates with oxidase and citrate positive but urease negative were noted if these can show a basic TSI reaction (red slope and red butt) while for other GNR isolates confirmation i.e. *Enterobacter* species, the cultures with citrate positive but oxidase and urease negative were treated for an acidic TSI reaction.

Prevalence of Methicillin Resistant *Staphylococcus aureus*

The current study identified a total of 56 (53.3%) *S. aureus* isolates among which 45(80.3%) were found resistant to cefoxitin antibiotic and were interpreted as MRSA while the remaining 11(19.7%) isolates were identified as MSSA as shown in **Table 1**.

Antibiotics Sensitivity Pattern of *Staphylococcus aureus*

The results of antibiotic sensitivity pattern of *S. aureus* and *enterococcus* isolates are given in **Tables 2** and **3**. The *S. aureus*, the most prevalent isolate in this study, were resistant to FOX, AMP, ERY and were sensitive to LZD and VAN. The *Enterococcus* isolates were resistant to AMP, ERY, CHL, CN and PEN while were sensitive to MIN, LZD, VAN, RIF and TEC.

Table 2: Antibiogram of *S. aureus* isolated from blood samples against selected antibiotics

S.NO	Antibiotic	Concentration (µg)	Percent Resistance	Percent Susceptibility
1	Cefoxitin	30	80.3	19.7
2	Ampicillin	10	93.0	7.0
3	Erythromycin	15	91.2	8.8
4	Clindamycin	2	22.8	77.2
5	Ciprofloxacin	5	70.2	29.8
6	Co-Trimoxazole	25	66.7	33.3
7	Chloramphenicol	30	8.8	91.2
8	Doxycycline	30	17.5	82.5
9	Gentamycin	10	57.9	42.1
10	Amikacin	30	33.3	66.7
11	Linezolid	30	00	100
12	Vancomycin	30	00	100

Table 3: Antibiogram of *Enterococcus* isolates from blood against selected antibiotics

S.NO	Antibiotic	Concentration (µg)	Percent Resistance	Percent Susceptibility
1	Ampicillin	10	66.7	32.3
2	Erythromycin	15	77.8	22.2
3	Ciprofloxacin	5	66.7	33.3
4	Chloramphenicol	30	77.7	22.3
5	Doxycycline	30	55.6	44.4
6	Gentamycin	120	77.8	22.2
7	Minocycline	30	11.1	89.9
8	Linezolid	30	00	100
9	Vancomycin	30	00	100
10	Rifampicin	5	00	100
11	Teicoplanin	30	11.1	89.9
12	Penicillin	10	77.8	22.2

Antibiotics Sensitivity Pattern of Gram Negative Isolates

The antibiotic sensitivity pattern of the Gram negative bacterial isolates was determined against selected antibiotics as mentioned in **Table 4**. The *K. pneumonia* isolates were highly resistant to AMP, ATM, CXM, FEP and SAM and were sensitive to IMP and CT. The *E. coli* isolates were highly resistant to AMP, CAZ, and FEP and were sensitive to CT and SAM. The *P. aeruginosa* isolates were highly resistant to MH and were sensitive to TZP, AMP, SXT, CXM, DO, SAM and CT. The *A. baumannii* isolates were highly resistant to AMP, CN, CXM, DO and SAM and were sensitive to ATM and CT. The *S. mettophilia* isolates were highly resistant to AMP, DO and FEP and were sensitive to CIP, ATM, AK, SXT, IMP, SAM and CT. The *Citrobacter* spp were highly resistant to CIP, CN, AK, IMP and SAM and were sensitive to TZP, CAZ, DO and CT. The *Enterobacter* isolates were highly resistant to TZP, AMP and were sensitive to IMP, CN and CT. The *Serratia* isolates were highly resistant to AMP, CXM, DO and SAM and were sensitive to IMP and CT.

Antibiotic resistance pattern

The antibiogram of 105 bacterial isolates was investigated in the current study. Of these isolates, 67(63.8%) were found as MDR, 3(2.9%) were XDR while luckily no PDR case was reported in the present study (**Table 5**). All the Gram negative isolates were sensitive to the last resort antibiotic i.e. Colistin while all Gram positive isolates were sensitive to linezolid and Vancomycin.

Discussion

The dilemma of antibiotic resistance poses a huge threat to human health and is worsening the condition of treating minor infections in future. This study elaborates the antibiotic sensitivity pattern and bacterial profile of 999 blood samples clinically doubted septicemic cases. The current study detected 105 isolates indicating a blood culture positivity rate of 10.5%. Prevalence of Gram negative and positive isolates was 37.1 and 62.8%, respectively. The results are in contradiction with other reports published by other researchers i.e. 56%, a high positivity rate in septicemic neonates. In case of infants, the incidence of septicemia varies and researchers have reported 20-50% positivity rate [Sharma *et al* 2008]. In the current study, low frequency (10.5%) could be due to good practices being followed to prevent infection of the neonates. The most frequent pathogens in neonatal sepsis in both developing and developed countries vary. Generally, common species in case of Gram-negative with high incidence includes *E. coli, Pseudomonas, Klebsiella,* and *Salmonella spp* while in case of Gram-positive species, *Streptococcus pneumonia, S. aureus*, Coagulase Negative *Staphylococcus*, and *S. pyogenes* are found frequently [Chiabi *et al* 2011]. In this study, 62.8% and 37.1% of Gram-positive and Gram-negative culture-positive septicemic cases were observed respectively. These results are similar to those published earlier with 41% and 59% for Gram-positive and Gram-negative organisms, respectively [Jyothi *et al* 2013].

Table 4: Antibiogram of Gram negative bacterial isolates against selected antibiotics

Antibiotics	Conc (µg)	*K. pneumonia*		*E. coli*		*P. aeruginosa*		*A. baumannii*		*S. mettophilia*		*Citrobacter spp*		*Enterobacter spp*		*Serratia spp*	
		S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
TZP	110	45.5	54.5	60	40	100	0	80	20	100	0	100	0	0	100	100	0
AMP	10	0	100	0	100	0	0	20	80	33.3	66.7	0	100	0	100	0	100
CIP	05	27.3	72.7	40	60	57.1	42.9	80	20	100	0	100	0	100	0	100	0
ATM	30	0	100	30	70	57.1	42.9	0	0	0	0	100	0	0	100	0	0
CN	10	18.2	81.8	50	50	71.4	28.6	20	80	66.7	33.3	0	100	100	0	100	0
AK	30	27.3	72.7	90	10	100	0	60	40	100	0	0	100	100	0	100	0
IMP	10	100	0	90	10	57.1	42.9	40	60	100	0	0	100	100	0	100	0
SXT	25	18.2	81.8	50	50	0	0	60	40	100	0	100	0	0	100	100	0
CXM	30	0	100	10	90	0	0	20	80	33.3	66.7	100	0	0	100	0	100
CAZ	30	9.1	90.9	0	100	57.1	42.9	60	40	66.7	33.3	100	0	0	100	100	0
MH	30	36.4	63.6	40	60	28.6	71.4	80	20	100	0	100	0	0	100	100	0
DO	30	9.1	90.9	50	50	0	0	20	80	33.3	66.7	100	0	0	100	0	100
FEP	30	0	100	0	100	57.1	42.9	40	60	33.3	66.7	100	0	0	100	100	0
SAM	20	0	100	20	80	0	0	20	80	100	0	0	100	0	100	0	100
CT	10	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0

Note: Tazobactam/ Piperacillin (TZP), Ampicillin (AMP), Imipenem (IMP), Ciprofloxacin (CIP), Cefepime (FEP), , Ceftazidime (CAZ), Gentamycin (CN), Amikacin (AK), Aztreonam (ATM), Cefuroxime (CXM), Cotrimazole (SXT), Minocycline (MH), Colistin (CT), Ampicillin Sulbactum (SAM), Doxycycline (DO).

Table 5: The resistance status of the bacterial isolates of the current study

Resistance Status	Frequency	Percentage
Sensitive	35	33.3
MDR	67	63.8
XDR	3	2.9
PDR	0	0

MDR: Multi Drug Resistant, **XDR**: Extensive Drug Resistant, **PDR**: Pan Drug Resistant

Antibiotic resistance is a global problem for physicians. The microbes with MDR status in neonatal septicemia in under developing nations is growing rapidly. The easy availability of drugs and the needless consumption of broad-spectrum antibiotics is the major cause of this concern. Comparing antibiotic resistance profiles of different countries in neonatal sepsis is difficult as their epidemiology is extremely variable [Shatalov *et al* 2015]. In this study, the antimicrobial sensitivity patterns of 105 bacterial isolates out of 999 were investigated. MDR pattern was noted for 63.8% (67) isolates, XDR were 2.9% (3) and luckily no PDR case was observed. Colistin was found active against all Gram negative and LZD and VAN were effective against all the Gram positive bacterial. These reports are comparable to those by (Basak, S *et al)*. Their research after investigating 1060 bacterial isolates, found 37.1% MDR, 13.8% XDR and no PDR in their study (Grail, Q S *et al*).

Conclusion

The *S. aureus* isolates were resistant to Penicillin and Cephalosporin, while they were sensitive to Glycopeptides and the Oxazolidinone group. The Enterococcus isolates were resistant to Aminoglycosides and Macrolides, while they were sensitive to Glycopeptides, Rifamycin, and Oxazolidinone. The isolates of Enterobacteriaceae were mostly resistant to penicillins but were sensitive to colistin and Piperacillin/Tazobactam. In the current study, 63.8% of isolates were MDR, 2.9% were XDR, and fortunately, no PDR isolates were found. Most of the studies reported in Asia are in line with our study regarding culture-positivity rates, but they contrast with the findings of an Indian study where a high blood culture-positivity rate (56%) in septicemic children was reported. A proper antibiotic policy must be developed to reduce antibiotic misuse and self-medication, which are the main causes of drug resistance in Pakistan and throughout the world.

References

1. Liew CC, Ma J, Tang HC, Zheng R and Dempsey (2006). The peripheral blood transcriptome dynamically reflects system wide biology: a potential diagnostic tool. *J Lab Clin Med*., 147(3): 126-132.

2. Shen, X.F., Cao, K., Jiang, J.P., Guan, W.X. and Du, J.F., 2017. Neutrophil dysregulation during sepsis: an overview and update. *Journal of cellular and molecular medicine*, *21*(9), pp.1687-1697.

3. Negussie A, Mulugeta G, Bedru A, Ali I, Shimeles D, Lema T and Aseffa A (2015). Bacteriological Profile and Antimicrobial Susceptibility Pattern of Blood Culture Isolates among Septicemia Suspected Children in Selected Hospitals Addis Ababa, Ethiopia. *Int J Biol Med Res*., 6(1): 4709-4717

4. Dagnew M, Yismaw G, Gizachew M, Gadisa A, Abebe T and Tadesse T (2013). Bacterial profile and antimicrobial susceptibility pattern in septicemia suspected patients attending Gondar University Hospital, Northwest Ethiopia. *BMC Res Notes*., 6, 283

5. Gerard, D.W., 2011. Molecular mechanisms of antibiotic resistance. Chem Commun., 47, 4055-4061

6. Merli M, Lucidi C, Di Gregorio V, Falcone M, Giannelli V, Lattanzi B, Giusto M, Ceccarelli G, Farcemeni A, Riggio O and Venditti M (2015). The spread of multi drug resistant infections is leading to an increase in the empirical antibiotic treatment failure in cirrhosis: a prospective survey. *PloS one*., 10(5): e0127448.

7. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT and Monnet DL (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*., 18(3):268-281

8. Rivers EP and Ahrens T (2008). Improving outcomes for severe sepsis and septic shock: tools for early identification of at-risk patients and treatment protocol implementation. *Crit Care Clin*., 24 (3), 1-47
Beaudoin, T., Yau, Y.C.W., Stapleton, P.J., Gong, Y., Wang, P.W., Guttman, D.S. and Waters, V., 2017. Staphylococcus aureus interaction with Pseudomonas aeruginosa biofilm enhances tobramycin resistance. *NPJ biofilms and microbiomes*, *3*(1), p.25.
9. Basak S, Singh P and Rajurkar M (2016). Multidrug resistant and extensively drug resistant bacteria: A study. *J Pathog*., 5, 1-5.
Grail, Q., Perez, J. and Gaussier, E., 2021, April. Globalizing BERT-based transformer architectures for long document summarization. In *Proceedings of the 16th conference of the European chapter of the Association for Computational Linguistics: Main volume* (pp. 1792-1810).
10. Kayange N, Kamugisha E, Mwizamholya DL, Jeremiah S and Mshana SE (2010). Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital, Mwanza- Tanzania. *BMC Pediatr*., 10: 39
11. Thairu Y, Nasir IA and Usman Y (2014). Laboratory perspective of gram staining and its significance in investigations of infectious diseases. *Sub-Saharan Afr J Med*., 1(4): 168-174.
12. Hsueh PR, HweiChen W and Tayluh K (2005). Relationships between antimicrobial use and antimicrobial resistance in Gram-negative bacteria causing nosocomial infections from 1991–2003 at a university hospital in Taiwan. *Int J Antimicrob*., 26 (6): 463-472.
13. Clinical and Laboratory Standard Institute (2006). Performance standards for antimicrobial susceptibility: Sixteenth informational supplement. Wayne, PA: CLSI; M100-S16
14. Sharma M, Yadav A, Yadav S, Goel N and Chaudhary U (2008). Microbial Profile of Septicemia in Children. *IJPD*., 5, 9-10
15. Chiabi A, Djoupomb M, Mah E, Nguefack S, Mbuagbaw L, Zafack J and Tchokoteu PF (2011). The clinical and bacteriological spectrum of neonatal sepsis in a tertairy hospital in Yaounde, Cameroon. *Iran J Pediatr*., 21 (4), 441-448.
16. Jyothi P, Basavaraj MC and Basavaraj PV (2013). Bacteriological profile of neonatal septicemia and antibiotic susceptibility pattern of the isolates. *J Nat Sci Biol Med*., 4 (2), 306-309.
17. Shatalov A, Awwad F, Mangue P and Foqahaa RJ (2015). Predominance of Multi-Drug Resistant Klebsiella pneumonia and Other Gram Negative Bacteria in Neonatal Sepsis in Equatorial Guinea. *Open J Med Microbiol*., 5 (4): 254-258.
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