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Original Article

The Commonly Encountered Bacterial Agents and their Antibiotic Sensitivity in the Neonatal Intensive Care Units of Cairo University Children Hospitals: An Observational Study

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Abstract:
Background: The emergence of multidrug-resistant organisms (MDROs) endangered neonates in the neonatal intensive care units (NICUs).
Aim of the work: To identify the most common bacterial agents and MDROs in neonatal sepsis in our center, their antibiotic sensitivity, the possible associated neonatal and maternal risk factors, and their relation to the outcome.
Subjects and Methods: This retrospective study included 138 neonates with culture-proven sepsis (blood, endotracheal tube aspirates (ETA), urine, cerebrospinal fluid (CSF), or wound swabs) admitted in 1 year (2022). Data was obtained from the patient's files, including various clinical and laboratory characteristics and risk factors and outcomes.
Results: This study included 138 newborns with confirmed neonatal sepsis. Of all cultures performed, multidrug resistant (MDR) *Klebsiella pneumoniae* was the most frequently isolated organism among 37 (27%) cases, that was mostly sensitive to polymyxin B was, followed by coagulase-negative *Staphylococcal aureus* (CoNS) in 32 cases, which was the most sensitive to vancomycin. Of all performed cultures, 71(51%) revealed MDROs (MDR *Klebsiella pneumoniae*, MRSA, MDR *Acinetobacter*, MDR *Enterobacter,* and MDR *E. coli*). The frequency of MRSA and MDR *Klebsiella pneumoniae* in the blood cultures was higher among those who died than those who survived (p= 0.03) and (p= 0.04) respectively. Logistic regression showed that bulging fontanel, feeding intolerance, temperature instability, long duration of admission, and high CRP were associated with high mortality among the studied cases (odd ratio > 1 (p= 0.0001), (p=0.007), (p=0.03), (p=0.001) and (p=0.006) respectively, while normal blood pressure, blood transfusion, appropriate birth weight, and normal serum albumin were protective variables (odd ratio < 1) (p= 0.008), (p=0.03), (p=0.001) and (p=0.001) respectively.
Conclusion: MDROs were isolated in more than 50% of neonatal sepsis. The methicillin-resistant *Staphylococcus aureus* and MDR *Klebsiella pneumoniae* in blood cultures were associated with higher mortality in neonatal sepsis.

Keywords: Neonatal sepsis; multidrug-resistant organisms; antimicrobial resistance; antibiotics; blood cultures
Abbreviations: CBC: Complete blood count; CoNS: coagulase-negative *Staphylococcal aureus*; CRP: C-reactive protein; CSF: cerebrospinal fluid; ETA: endotracheal tube aspirates; IMCI: Integrated Management of Childhood Illness; MDR Multidrug-resistant; MRSA: methicillin-resistant *Staphylococcal aureus*; NEC: necrotizing enterocolitis; NICUs: neonatal intensive care units; PCR: polymerase chain reaction; PROM: premature rupture of membranes; TPN: total parenteral nutrition; UTI: urinary tract infection; WHO: World Health Organization

Introduction

Newborns admitted to the neonatal intensive care units (NICUs), especially preterm newborns, are highly susceptible to infections, which may progress rapidly due to their immature immune systems (*1*). The neonatal susceptibility to infection increases with exposure to various intrapartum and postpartum risk factors. Blood culture is the gold standard for diagnosis of neonatal sepsis. It may be affected by empiric antibiotic use and the small volume of the obtained sample from the tiny newborns but unfortunately. Compliance to various guidelines is necessary for optimal thriving of bacterial culture and avoid the risk of contamination (*2*). Blood culture may take a few days to yield. Hence, retrospective identification of the most frequent pathogens,

allow anticipation of the type of pathogen, and empiric specific antibiotic prescription (*3*). Antimicrobial agents are frequently prescribed in the NICUs. The choice of antibiotics is crucial; it depends on the clinical presentation and the suspected organism; this choice is widely variable due to the lack of standard practice guidelines for escalating the antibiotic regimen (*4*). Antibiotic resistance is a global significant threat (*5*). The prevalence of multidrug-resistant organisms is increasing, endangering human lives, especially in the NICUs (*6*). In the present study, we aimed to detect the most common bacterial agents infecting neonates in our center, their antibiotic sensitivity, the possible neonatal and maternal risk factors associated with neonatal septicemia, and their relation to the outcome.

Subjects and Methods

This retrospective study was conducted at the Neonatal Intensive Care Units (NICUs) of Cairo University Children's Hospitals during 12 months (January 2022- December 2022). The Ethical Committee of the Faculty of Medicine, Cairo University, Egypt, approved the study (Approval Code: MS-22-2021).

Participants

One thousand four hundred ninety-three neonates were admitted to Cairo University NICUs throughout the year 2022; of them 278 were suspected to have neonatal sepsis during admission based on the international guidelines for the management of sepsis and septic shock 2021 (*7*); different types of cultures were performed, according to the clinical manifestations, including blood, endotracheal aspirate (ETA), urine, cerebrospinal fluid (CSF) and wound swab cultures. Those with positive cultures were 138, were included in the study. We excluded files of premature neonates with gestational age less than 28 weeks, those admitted with congenital pneumonia i.e., neonates presenting within 1 week of birth with respiratory manifestations, with chest X-ray showing early infiltrates and areas of consolidation and followed by isolation of the organism**,** as well as those with negative cultures.

Methods

The collected data from the patient's files included: 1) History about maternal risk factors for sepsis such as offensive liquor, prolonged rupture of membranes (PROM) >18 hours, maternal fever >38°C, maternal urinary tract infection (UTI) and history of TORCH infection, other maternal risk factors such as gestational hypertension, and gestational diabetes. 2) Natal history including gestational age, mode of delivery, and obstructed labor. 3) Post-natal history including birth weight, history of severe apnea, bradycardia or arrest, respiratory distress, hypoactivity. 4) Admission history including the duration of hospital stay, the medical interventions required such as endotracheal tube insertion, mechanical ventilation, total parenteral nutrition (TPN), central venous umbilical catheter, and need for phototherapy or exchange transfusion, and the outcome.

Data of clinical examination included the assessment of gestational age through Ballard score (*8*), maternal dates and the assessment of the clinical manifestations of sepsis, including the general condition of the newborns, the vital signs, the perfusion status, presence of sclerema, respiratory signs (severe apnea or respiratory distress), gastrointestinal signs (feeding difficulties, abdominal distention, and suspected necrotizing enterocolitis (NEC)), central nervous system signs (convulsions, drowsiness, unconsciousness, decreased activity or bulging fontanel). Data of the performed investigations included the complete blood count (CBC) with the differential leucocytic count, C-reactive protein (CRP), different types of cultures, including blood, endotracheal aspirate (ETA), urine, cerebrospinal fluid (CSF), and wound swab cultures, their sensitivity, polymerase chain reaction (PCR) swabs for COVID-19 if available and chest X-ray. Data of the received treatment were also recorded, the antibiotics given to neonates and their responses. Patients were divided into two groups according to the outcome: Group (1) included those who survived (n=106), and Group (2) included those who died (n=32).

Statistical Analysis

We used Statistical Package for Social Sciences version 24 for Windows (SPSS, IBM, USA). We used frequencies and percentages to describe the qualitative variables. For numerical variables, we used mean and standard deviations for parametric data. We used the Shapiro-Wilk test to test the normality of numerical variables. We used Spearman correlation to test the correlation between numerical variables. We used repeated measures ANOVA to test the difference between more than two numerical variables for parametric data and the Friedman test for nonparametric data. A p-value of less than 0.05 was considered statistically significant.

Results

This study included 138 newborns; 79 (57 %) were males, 82 (59%) were preterm (29-38 weeks), and 113 (82%) were delivered by Cesarean section. Of them, 106 (76.8%) survived and were discharged, while 32 (23.2%) died. (Figure 1). The mean ± SD age at admission was 4.5 ±5 days (range= 1-25 days) with a median and IQR= 2(1-4) days. The mean± SD weight of the studied newborns was 2.4 ± 0.7 kg, while the mean± SD duration of hospital stay was 25 ± 15 days. The indications of admission and risk factors for sepsis are outlined in Table 1.

Table 1. Clinical characteristics of the studied neonates, maternal risk factors, and indication of admission

	Neonatal Sepsis Cohort		**Group 1 (Survivors)**		**Group 2 (Died)**		**P value**
	N=138	%	N=106	%	N=32	%	
Sex							
Male	79	57	63	57	15	47	0.32
Female	59	43	43	41	17	53	
Maturity							
Preterm	82	59	57	54	24	75	**0.003**
Full term	56	41	49	46	8	25	
Mode of delivery							
Cesarean section	113	82	86	81	27	84	0.6
Normal vaginal delivery	25	18	20	19	5	16	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	
Age in days	4	5	5	5	3	4	**0.00**
Weight in kgs	2.5	0.7	2.46	0.65	1.72	0.76	**0.000**
Admission duration (days)	25	15	27	17	19	10	**0.008**
Maternal risk factors	**Number**	**%**	**Number**	**%**	**Number**	**%**	
PROM	32	23	24	23	8	25	0.07
Maternal hypertension	17	12.3	14	13	3	9	0.5
COVID-19 positive mothers	9	6.5	8	7.5	1	3	0.4
Antepartum hemorrhage	8	5.7	6	5.6	2	6	0.9
Maternal diabetes mellitus	7	0.5	6	5.6	1	3	0.6
Oligohydramnios	2	1.4	1	0.9	1	3	0.4
Chronic renal failure	1	0.7	1	0.9	0	0	0.6
Rheumatic heart disease	2	1.4	1	0.9	1	3	0.4
Urinary tract infection	1	0.7	1	0.9	0	0	0.6
Indication of admission	**Number**	**%**	**Number**	**%**	**Number**	**%**	
Respiratory distress	79	57	53	50	26	81	**0.002**
Refusal of feeding	15	9.4	15	12	0	0	**0.02**
Neonatal jaundice	11	7.9	11	10	0	0	0.06
Cyanosis	7	5	5	4.7	2	6	0.73
Convulsions	8	5.8	7	6.6	1	3	0.46
Acute kidney injury	5	3.6	3	2.1	2	6	0.36
Urinary tract infection	4	2.9	4	3.7	0	0	0.26
Fever	2	1.4	2	1.8	0	0	0.6
Congenital anomalies	3	2.1	2	0.9	1	3	0.67
Necrotizing enterocolitis	2	1.4	2	1.8	0	05	0.6
Thrombophilia	1	0.7	1	0.9	0	0	0.58
Hypoglycemia	3	2.1	3	2.8	0	0	0.33
Interventions	**Number**	**%**	**Number**	**%**	**Number**	**%**	
Endotracheal intubation	87	68	58	54	29	90	**0.000**
Umbilical catheterization	61	44	43	40	18	56	0.1
Total parenteral nutrition	67	49	43	40	24	75	**0.000**
Blood transfusion	45	32	41	37	6	19	**0.045**
Peritoneal dialysis	3	2.1	2	2	1	3	0.681

PROM: premature rupture of membranes

Poor activity, drowsiness, and feeding intolerance were the most commonly presenting clinical presentations. 41(30%) of the neonates suffered from hypotension, and 28 (20%) had tachypnea.

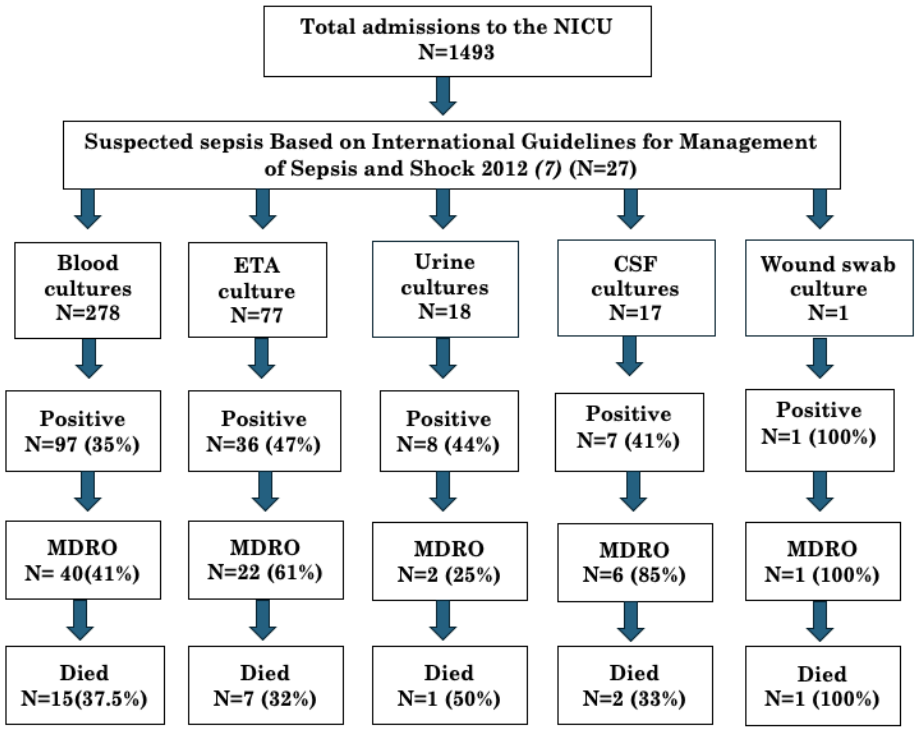

Figure 1. Flow chart of the study population
ETA: endotracheal aspirate; CSF: cerebrospinal fluid.

Anemia was present in 78(56.5%) of studied neonates, 152 (54.7%) neonates had thrombocytopenia, 56 (40.5%) had leucopenia and only 1 (0.7%) had leukocytosis. Lymphopenia was encountered in 24 (17.3%) patients. In contrast, lymphocytosis was found only in 1 (0.7%) patient, neutropenia was found in 20 (14.5%) of the studied neonates, CRP was positive in 132 (95.6%) of the cases with a mean ± SD of 49 ± 32 (mg/L). (Table 2). Among our cohort 127 (92%) had positive cultures from a single site, 11(8%) had positive cultures from 2 sites, and none of our patients had positive cultures from 3 or 4 sites. Coagulase-negative *Staphylococcus aureus* (CoNS) was the commonest isolated organism from blood cultures in 31 (32%) patients, MDR *Klebsiella pneumoniae* was the most common organism in ETA cultures in 17 (47%), MDR *E. coli* and *Candida albicans* were the most frequently isolated organisms in urine cultures, both had a frequency of 3 (37.5%), and MDR *Klebsiella pneumoniae* was the most isolated from cerebrospinal fluid culture (CSF) cultures with a frequency of 4 (57%). One patient only required a wound swab culture (from a peritoneal catheter wound), which revealed MDR *Klebsiella pneumoniae,* that was sensitive to ciprofloxacin, levofloxacin, and polymyxin B. [Table 3].

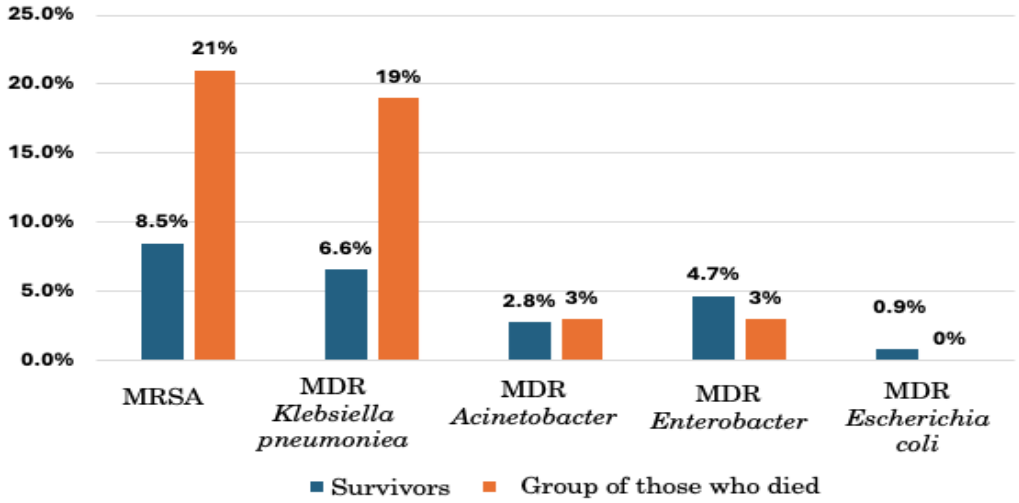

Figure 2. Frequency of MDR organisms in blood cultures among neonates who survived and those who died

Multidrug-resistant *Klebsiella pneumoniae* was the most frequently isolated organism -in 37 (27%) cultures-, which was highly sensitive to polymyxin B, followed by CoNS which was highly sensitive to vancomycin. More than half of all performed cultures 71 (51%), revealed multidrug-resistant organisms (MDR *Klebsiella pneumoniae*, *MRSA*, MDR *Acinetobacter*, MDR *Enterobacter,* and MDR *E. coli*). (Table 3).

Table 2. Clinical manifestations and laboratory investigations of the studied neonates

	All Neonatal Sepsis		**Group 1 (Survivors)**		**Group 2 (Died)**		**P value**
	N=138	%	N=106	%	N=32	%	
Poor activity	116	84	86	81	30	94	0.1
Drowsiness	66	48	47	44	19	59	0.13
Bulging anterior fontanel	16	12	7	7	9	28	**0.009**
Convulsions	17	12	10	9.4	7	22	0.06
Feeding intolerance	55	40	40	38	25	78	**0.000**
Abdominal distention	46	33	27	25	19	59	**0.000**
Diarrhoea	2	1.4	0	0	2	6	**0.009**
Periumbilical erythema	6	4.3	3	2.2	3	9	0.11
Oedema over joint	20	14.4	12	11	8	25	0.05
Skin pustules	2	1.4	0	0	2	6	**0.009**
Temperature instability	39	28	22	21	17	**53**	**0.000**
Delayed CRT	42	30	22	20	18	18	**0.000**
Hypotension	41	30	26	24	15	15	**0.01**
Tachypnoea	28	20	14	13	14	14	**0.000**
	Mean	SD	Mean	SD	Mean	SD	
Heart rate (beat /minute)	149	12	148	11	154	13	**0.01**
Systolic BP	73	8	75	7.5	69	9	**0.01**
Diastolic BP	45	9	45	9	42	10	0.06
Respiratory rate (breath/minute)	50	7	49	7	55	7	**0.00**
Hemoglobin (g/dl)	12	2	12	2.2	13	2.5	0.05
Platelets (10^3/cmm)	207	99	216	99	176	99	**0.048**
Total leucocytic count (10^3/cmm)	11.5	5.7	11.4	5.4	12	7	0.7
Absolute neutrophil count (cell/µL)	5697	3816	5526	3745	6265	4167	0.34
Absolute lymphocyte count (cell/µL)	3615	2051	3575	1714	3746	2980	0.7
I/T ratio	0.18	0.09	0.17	0.09	0.2	0.1	0.24
C reactive protein (mg/dl)	49	32	45	31	63	34	**0.005**
Urea (mg/dl)	36	19	34	15	43	28	**0.03**
Creatinine (mg/dl)	0.5	0.4	0.4	0.3	0.7	0.5	**0.00**
ALT (U/L)	24	17	24	18	22	13	0.5
AST (U/L)	46	19	45	19	49	20	0.3
Serum Albumin (g/dl)	2.9	0.41	3	0.4	2.8	0.3	**0.004**
TSB (mg/dl)	5.6	8.9	6	9	4	3.4	0.2
DSB (mg/dl)	0.5	0.9	0.6	1	0.3	0.4	0.1

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BP: blood pressure; CBC: complete blood count; CRT: capillary refill time; DSB: direct serum bilirubin; GIT: Gastrointestinal tract; I/T ratio: immature-to-total leucocytic count; TSB: total serum bilirubin.

The frequency of MRSA and MDR *Klebsiella pneumoniae* in the blood cultures was higher among those who died than those who survived (p=0.03) and (p=0.04), respectively), while the frequency of other multidrug-resistant organisms did not differ between both groups. (Table 5, Figure 2).

Tables 1 and 2 summarize the risk factors that were associated with mortality. Group 2 (those who died) had statistically significant higher frequency of prematurity, bulging fontanel, feeding intolerance, abdominal distention, hypotension, temperature instability, delayed capillary refill time (CRT), tachypnea, grunting, the need for endotracheal intubation, umbilical catheterization and total parenteral nutrition (TPN) (p= 0.05). Logistic regression showed that bulging fontanel, feeding intolerance, temperature instability, long duration of admission, and elevated CRP were associated with 4.3, 3.7, 1, and 1 folds of rise (p= 0.0001), (p=0.007), (p=0.03), (p=0.001) and (p=0.006) respectively. In contrast, normal blood pressure, blood transfusion, appropriate birth weight, and serum albumin were associated with 0.4, 0.4, 0.2 and 0.7 lower risk folds and higher survival rate among the studied cases (odd ratio <1) (p= 0.008, (p=0.03), (p=0.0001) and (p=0.001) respectively. (Table 6).

Table 3. Analysis of results of the blood, endotracheal tubes aspirate, Urine, and cerebrospinal fluid culture

Organism	Blood culture (n=97)		ETA cultures (n=36)		urine cultures (n=8)		CSF cultures (n=7)		Total (n=138)		Most sensitive antibiotic
	No	%	No	%	No	%	No	%	No	%	
CoNS	31	32	0	0	0	0	1	14	32	23	Vancomycin
MRSA	16	16.4	0	0	0	0	1	14	17	12	Vancomycin
Klebsiella pneumoniae	13	13.4	6	16.6	1	12.5	0	0	20	15	Polymyxin
MDR *Klebsiella pneumoniae*	13	13.4	17	47	2	25	4	57	37	27	Polymyxin
MDR *Enterobacter*	6	6.1	1	2.7	0	0	0	0	7	5	Polymyxin
MDR *Acinetobacter*	4	4.1	3	8.3	0	0	1	14	8	6	Polymyxin
Pseudomonas aeruginosa	3	3	7	19	0	0	0	0	10	7	Amikacin Imipenem Ciprofloxacin
Hemolytic streptococci	3	3	0	0	0	0	0	0	3	2	Linezolid Doxycycline
Enterococci	2	2	0	0	0	0	0	0	2	1.4	Vancomycin Nitrofurantoin
Candida albicans	2	2	0	0	3	37.5	0	0	5	3.6	Ketoconazole
Acinetobacter	1	1	1	2.7	0	0	0	0	2	1.4	Polymyxin
Stenotrophomonas	1	1	0	0	0	0	0	0	1	0.7	Vancomycin
Escherichia coli	1	1	0	0	3	37.5	0	0	4	3	Levofloxacin
MDR *Escherichia coli*	1	1	1	2.7	0	0	0	0	2	1.4	Imipenem Tigecycline

CoNS: coagulase-negative *Staphylococcal aureus*; CSF: cerebrospinal fluid culture; ETA: endotracheal tubes aspirate; MDR: multi-drug resistant; MRSA: methicillin-resistant Staphylococcus aureus

Table 4. The various isolated organisms according to outcome

Culture	Organism	Group 1 (Survivors) N = 106	Group 2 (Died) N= 32	P value
Blood	Methicillin Resistant *Staphylococcus aureus*	9 (8.5%)	7(21%)	**0.03**
	MDR *Klebsiella pneumoniae*	7 (6.6%)	6 (19%)	**0.04**
	MDR *Acinetobacter*	3 (2.8%)	1 (3%)	0.95
	MDR *Enterobacter*	5(4.7%)	1 (3%)	0.67
	MDR *Escherichia coli*	1 (0.9%)	0 (0%)	0.58
	CONS	26(24%)	5(16%)	0.3
	Klebsiella pneumoniae	12(11%)	1(3%)	0.16
	Stenotrophomonas	1(0.9%)	0(0%)	0.58
	Escherichia coli	1(0.9%)	0(0%)	0.58
	Pseudomonas aeruginosa	2(1.8%)	0(0%)	0.43
	Candida albicans	2(1.8%)	0(0%)	0.43
	Haemolytic streptococci	3(2.8%)	0(0%)	0.33
	Enterococci	2(1.8%)	0(0%)	0.43
ETA	MDR *Klebsiella pneumoniae*	12 (11%)	5 (16%)	0.51
	MDR *Acinetobacter*	3 (2.8%)	0 (%)	0.17
	MDR *Enterobacter*	0 (0%)	1 (3%)	0.07
	MDR *E. coli*	0 (0%)	1 (3%)	0.07
	Klebsiella pneumoniae	5(4.7%)	1(3%)	0.7
	Pseudomonas aeruginosa	5(4.7%)	2(6%)	0.73
	Enterococci	0(0%)	1(3%)	0.07
	Acinetobacter	1(0.9%)	0(0%)	0.58
Urine	*Klebsiella pneumoniae*	2(1.8%)	0 (0%)	0.43
	Escherichia coli	2(1.8%)	0 (0%)	0.43
	Candida albicans	3(2.8%)	0 (0%)	0.33
	MDR *Klebsiella pneumoniae*	0 (0%)	1 (3%)	0.07
CSF	MDR *Klebsiella pneumoniae*	2 (1.8%)	2 (6%)	0.2
	MDR *Acinetobacter*	1 (0.9%)	0 (0%)	0.58
	CoNS	1(0.9%)	0(0%)	0.58
	Methicillin Resistant *Staphylococcus aureus*	1(0.9%)	0(0%)	0.58
Wound swab	MDR *Klebsiella pneumoniae*	0 (0%)	1 (%)	0.07

CSF: cerebrospinal fluid culture; ETA: endotracheal tubes aspirate; MDR: multi-drug resistant

Table 5. Sensitivity and resistance of antibiotics in the different culture:

Antibiotics (in alphabetical order)	Blood culture (No=97)		ETA culture (No=36)		Urine cultures (No=8)		CSF culture (No=7)	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Amikacin	22 (23%)	41(43%)	7(19%)	13(36%)	2(25%)	3(38%)	0%	2(29%)
Amoxicillin	0%	41(43%)	0%	9(25%)	0%	3(38%)	0%	0%
Amoxicillin-clavulanate	0%	57(60%)	0%	0%	0%	4(50%)	0%	2(29%)
Amphotericin-B	0%	0%	0%	0%	1(12%)	2(25%)	0%	0%
Ampicillin	0%	61(64%)	0%	18(50%)	0%	3(38%)	0%	2(29%)
Ampicillin-sulbactam	1(1%)	39(40%)	1 (3%)	15(42%)	0%	4(50%)	0%	2(29%)
Azithromycin	5 (5%)	6(6%)	0%	0%	0%	2(25%)	0%	0%
Aztreonam	0%	11(12%)	0%	5(14%)	0%	3(38%)	0%	0%
Carbenicillin	0%	42(44%)	0%	9(25%)	0%	3(38%)	0%	0%
Cefaclor	0%	38(40%)	0%	9(25%)	0%	3(38%)	0%	0%
Cefazolin	0%	41(43%)	0%	9(25%)	0%	3(38%)	0%	0%
Cefipime	1 (1%)	36 (37%)	6(17%)	15(42%)	0%	4(50%)	0%	1(14%)
Cefixime	0%	38(40%)	0%	9(25%)	0%	3(38%)	0%	0%
Cefoperazone	0%	41(43%)	0%	9(25%)	0%	4(50%)	0%	0%
Cefotaxime	0%	51(53%)	0%	13(36%)	0%	4(50%)	0%	0%
Cefoxitin	1% (1)	63(64%)	0%	20(56%)	0%	4(50%)	0%	2(29%)
Ceftazidime	1 (1%)	41 (43%)	2(6%)	16(44%)	0%	4(50%)	0%	1(14%)
Ceftriaxone	1(1%)	46(48%)	2(6%)	17(47%)	0%	4(50%)	0%	2(29%)
Cefuroxime	0%	41(43%)	0%	16(44%)	0%	3(38%)	0%	2(29%)
Cephalexin	0%	40(42%)	0%	9(25%)	0%	3(38%)	0%	0%
Chloramphenicol	14 (15%)	2(2%)	0%	0%	0%	4(50%)	0%	0%
Ciprofloxacin	32 (33%)	30(31%)	12(33%)	10(28%)	5(62%)	2(25%)	0%	1(14%)
Clindamycin	17 (18%)	22(23%)	0%	0%	0%	4(50%)	1(14%)	0%
Doxycycline	21 (22%)	7 (7%)	0%	0%	0%	2(25%)	1(14%)	0%
Ertapenem	0%	4(4.2%)	0%	1(3%)	0%	2(25%)	0%	0%
Erythromycin	13(13%)	18(19%)	0%	0%	0%	2(25%)	1(14%)	0%
Fluconazole	0%	0%	0%	0%	0%	3(38%)	0%	0%
Gentamicin	0%	56(58%)	0%	19(53%)	1(12%)	4(50%)	0%	2(29%)
Imipenem	12(12%)	38% (37)	11(31%)	14(39%)	2(25%)	3(38%)	0%	2(29%)
Kanamycin	0%	2(2%)	0%	0%	0%	2(25%)	0%	0%
Ketoconazole	0%	0%	0%	0%	2(25%)	1(12%)	0%	0%
Levofloxacin	28(29%)	23 (24%)	10(28%)	5(14%)	3(38%)	2(25%)	2(29%)	0%
Linezolid	30 (32%)	2(2%)	0%	0%	0%	2(25%)	1(14%)	0%
Meropenem	7(7%)	36(37%)	1 (3%)	13(36%)	2(25%)	3(38%)	0%	2(29%)
Metronidazole	0%	7(7%)	0%	3(8%)	0%	3(38%)	0%	0%
Neomycin	0%	2(25%)	0%	0%	0%	2(28%)	0%	0%
Nitrofurantoin	2 (2%)	2(2%)	0%	0%	0%	2(28%)	0%	0%
Norfloxacin	0%	2(2%)	0%	0%	2(25%)	2(25%)	0%	0%
Ofloxacin	0%	7(7%)	0%	3(8%)	0%	2(25%)	0%	1(14%)
Penicillin	0%	41(43%)	0%	9(25%)	0%	3(38%)	0%	0%
Piperacillin	0%	42(44%)	0%	9(25%)	0%	3(38%)	0%	0%
Piperacillin-tazobactam	1(1%)	44(46%)	0%	20(56%)	0%	3(38%)	0%	2(29%)
Polymyxin	37 (39%)	2(2%)	20 (56%)	0%	3(38%)	2(25%)	6(86%)	0%
Rifampicin	9 (9%)	2(2%)	0%	0%	0%	4(50%)	0%	0%
Streptomycin	1(1%)	2(25%)	0%	0%	0%	2(25%)	0%	0%
Teicoplanin	6(6%)	2(2%)	0%	1(3%)	0%	2(25%)	0%	0%
Tetracycline	10 (10%)	10(10%)	0%	0%	0%	4(50%)	1(14%)	0%
Ticarcillin	0%	42 (44%)	0%	9(25%)	0%	4(50%)	0%	0%
Tigecycline	21 (22%)	3(3%)	16(44%)	0%	2(25%)	2(25%)	3(43%)	0%
Tobramycin	0%	2 (2%)	0%	0%	2(25%)	2(25%)	0%	0%
Trimethoprim-Sulphamethoxazole	23 (24%)	28 (29%)	1(3%)	16(44%)	0(0%)	4(50%)	0%	1(14%)
Vancomycin	47 (49%)	6(6.3%)	0%	0%	0%	2(25%)	2(29%)	0%

Data is represented in numbers and percentages.
CSF: cerebrospinal fluid; ETA: endotracheal tube aspirate.

The bacterial sensitivity and resistance to antibiotics were as follows: in blood cultures, isolated bacteria had highest sensitivity to vancomycin in 47(49%) cultures, followed by polymyxin B in 37(38.5%), while the isolated bacteria were resistant to cefoxitin in 63 (65.6%) and ampicillin in 61 (63.5%). In ETA cultures, isolated bacteria had highest sensitivity to polymyxin in 20 (55.6%) and tigecycline in 16 (44.4%) and the isolated bacteria were resistant to piperacillin-tazobactam in 20 (55.6%) and cefoxitin in 20 (55.6%). In urine cultures, isolated bacteria had highest sensitivity to ciprofloxacin in 5(62%), levofloxacin in 3 (38%) and polymyxin in 3 (38%) and the isolated bacteria were resistant to ampicillin-sulbactam, amoxicillin-

clavulanate, gentamicin ceftriaxone, trimethoprim-sulfamethoxazole, ceftazidime, cefepime, ticarcillin, cefoxitin, cefoperazone and cefotaxime. All were resistant in 4 (50%) of the cases. In CSF cultures, isolated bacteria had highest sensitivity to polymyxin in 6 (85.7%) and tigecycline in 3 (42.9%) and the isolated bacteria were highly resistant to ampicillin/sulbactam, amikacin, gentamycin, ceftriaxone meropenem, imipenem, piperacillin-tazobactam, ampicillin, amoxicillin-clavulanate, cefoxitin and cefuroxime. All were resistant in 2(28.6%) of the cases. (Tables 4, 5 and 6).

Table 6. Logistic Regression Risk for mortality among our studied cohort

	Odds ratio	Confidence interval for the odds ratio		P value
		Lower	Upper	
Maturity	0.62	0.28	1.41	0.26
Drowsiness	1.62	0.82	3.18	0.16
Bulging fontanelle	4.3	1.92	9.68	0.000
Feeding intolerance	3.7	1.43	9.79	0.007
Abdominal distension	1.2	0.58	2.66	0.57
Blood pressure	0.4	0.2	0.78	0.008
Capillary refill time	1.6	0.69	3.89	0.25
Grunting	1.4	0.26	7.72	0.69
Intubation	3.5	0.49	25.2	0.21
Umbilical catheterization	1.1	0.46	2.69	0.82
Peritoneal dialysis	1.7	0.71	4.02	0.23
Blood transfusion	0.4	0.17	0.91	0.03
Temperature instability	2.3	1.04	4.89	0.03
Age	0.9	0.83	1.02	0.12
Duration of admission	1.1	1.07	1.16	0.00
Weight	0.2	1.88	5.59	0.00
Hemoglobin	0.95	0.81	1.12	0.56
High C-reactive protein	1.01	1.06	1.18	0.006
Low Serum albumin	0.7	1.94	16.61	0.001
Reduced Platelets (10^3/cmm)	1.01	0.99	1.005	0.51

Discussion

The bacterial neonatal sepsis had a 23% fatality among our studied cohort in Cairo University NICU across Bacterial neonatal sepsis had a 23% fatality among our studied cohort in Cairo University NICU across the year 2022. The studied neonates had gestational age ranging from 29 to 38 weeks, PROM was the most frequently reported risk factors in 32(23%) of our cases followed by maternal hypertension in 17(12.3%). Measures should be taken to reduce the incidence of these risk factors, including reducing maternal reproductive system infections, and regular follow up visits to the gynecologists for timely proper treatment. Prematurity was significantly associated with mortality in our study; many previous studies have demonstrated the association of prematurity to the higher possibility of maternal cervical incompetence leading to PROM and intraamniotic fluid chorioamnionitis with subsequent neonatal sepsis (*9*).

The timely diagnosis of neonatal sepsis is difficult. Using the international guidelines for the management of sepsis and septic shock 2021(*7*); 49.6% were not confirmed by culture isolates. Procalcitonin testing is not a routine test among our studied cohort (*10*). The high culture negative suspected neonatal sepsis events ignite more questions than answers. This high burden of culture negative sepsis may represent the burden of viral infections, sepsis mimics, as metabolic, or respiratory distress syndrome, intestinal obstruction, cardiovascular conditions especially if leading to shock, or neonatal convulsions, etc. (*11*), hence the need for validation of this diagnostic scoring system in our center is underscored. And the need for neonatal screening for underlying metabolic disorders and family counseling is paramount. This high culture negative burden maybe related to pre-admission antibiotics; hence all antibiotic use should be recorded. If the etiology of the culture negative sepsis was the antibiotic use, then it seems that this empirical use was successful in controlling almost 50% of the infections and should be recognized and considered in future protocol guideline planning. We recommend studying the use of other methods to isolate organisms including PCR amplification or blood BIOFIRE for early and accurate bacterial isolation (*12*).

The MDR bacteria were responsible for most of the deaths. Despite the use of more recently developed classes of antibiotics as polymixin B and vancomycin, yet the fatality rate was high. The antimicrobials are widely prescribed in the NICUs, even pre-emptively, as the clinical manifestations of infection sometimes are indistinguishable from other non-infectious diseases (*3*). In most centers, broad-spectrum antibiotics are usually prescribed until the results of cultures are available. Unfortunately, numerous adverse effects may be encountered, such as gastrointestinal tract, renal effects (*13*), and, more importantly, the emergence of multidrug-resistant (MDR) organisms. There are no universal guidelines for implementing the antimicrobial regimens explicitly directed toward the challenges faced in the NICUs (*1*). Each center must have its peculiar map of bacterial distribution and effective antibiotics to make it easier for clinicians to choose and upgrade antibiotics while awaiting the results of cultures, which may take several days to appear. In our center, and many other centers, the combination of ampicillin-sulbactam and gentamicin is commonly used as a broad-spectrum antibiotic regimen till the results of blood culture are available (*14*). However, based on the present work, amikacin would be better as the bacterial isolates had higher sensitivity to it (23% vs. 0%) and less resistance rate (43% vs. 58%) compared to gentamicin among those with positive blood cultures (*15*). Annual analysis and revision of protocol antibiotics seems prudent to cope with the everchanging bacterial isolates.

The emergence of new strains of multidrug-resistant (MDR) organisms is a significant concern (*16*). Multidrug-resistant (MDR) *Klebsiella pneumoniae* was the most frequently isolated organism in the different cultures of our cohort. Previously, MDR *Klebsiella pneumoniae* was the most commonly isolated gram-negative MDR organism worldwide in neonatal sepsis (*5*) and also in previous studies from Egypt (*17*). It has been associated with several risk factors, such as prematurity, low birth weight, long duration of hospital stays, and the prolonged use of beta-lactam antibiotics and aminoglycosides (*18*). In our study, *Klebsiella pneumoniae* was most sensitive to was polymyxin. Still, we recommend restricting it's use to cases with proven MDR *Klebsiella pneumoniae* to guard against the emergence of new strains resistant to all polymyxin (*17*). In our study, the most isolated organism in the blood cultures was coagulase-negative *Staphylococcus aureus* (CoNS), in concordance with earlier reports, that showed that CoNS was the most frequently isolated organism associated with late-onset sepsis (*19*). Fortunately, it has been associated with low mortality (*18*). It has been related to factors violating the mucosal barriers, such as mechanical ventilators, central venous catheters, and parenteral nutrition (16). Methicillin-resistant *Staphylococcus aureus* (MRSA) was the most found multidrug-resistant organism in the blood cultures, and it was sensitive to vancomycin, which is widely used in the NICUs (*20*). Caution should be taken due to the emergence of some strains of vancomycin-resistant *Staphylococcus aureus* (*21*).

Healthcare-associated infections are serious problems that usually lead to fatal outcomes and longer duration of hospital stay with a massive burden on the economic status, especially in limited resources country (*22*), and more seriously lead to neurodevelopmental consequences in vulnerable newborns (*23*).

In the present work, the incidence of MRSA and MDR *Klebsiella pneumoniae* in the blood cultures was higher among those who died than those who survived (p-value 0.03 and 0.04, respectively); this emphasizes the tremendous need to exert more efforts to prevent the spreading of infections, especially multidrug-resistant organisms. Infection control measures include proper hand washing, optimum disinfection, the use of personal protective equipment, and sterilization policies. Also, increasing the nurse-to-patient ratio and continuous awareness among healthcare personnel regarding infection control measures is essential. Also, constant surveillance and revision of antibiotic regimens (*24*).

Intrapartum antibiotics for mothers with premature rupture of membranes (PROM) to reduce the incidence of neonatal sepsis, Close adherence to infection control measures during any procedure inside NICU, especially endotracheal intubation and blood transfusion, limiting the use of umbilical catheters, and starting oral intake as soon as possible in neonatal sepsis patients to shorten the duration of TPN are all recommended to decrease the consequences of neonatal sepsis.

The delivery rate by Caesarian section was 82%, which is alarming. Egypt has highest rates of C-section deliveries globally as announced by Egyptian Minster of Health (*25*). Caesarean section comes with its challenges, that might be related to this massive MDROs boom.

Limitations of the study include missing other sepsis biomarkers such as procalcitonin, interleukin-6, and interleukin-8. Future research is needed to discover new quick modalities for rapidly identifying organisms, such as polymerase chain reactions (PCR) or blood BIOFIRE, that may be a cost, time-saving, and accurate solution(11), thus limiting the empiric use of antibiotics.

Extensive and regular studies for reviewing microbial agents peculiar to each NICUs should be done regularly. Another study limitation is lack of long term follow up. We are not aware of the long-term complications of the bacteria or the antibiotics on the remodeling and future growth and development of these neonates.

Conclusion

Bacterial neonatal sepsis has high mortality. MDROs were isolated in more than half of all the performed cultures. MDR *Klebsiella pneumoniae* was the most frequently isolated organism, followed by coagulase-negative *Staphylococcal aureus (CoNS).* The frequency of methicillin-resistant *Staphylococcus aureus* and MDR *Klebsiella pneumoniae* in blood cultures was higher among newborns who died than those who survived. Fighting against the emergence of MDR organisms is necessary for better outcomes through proper infection control measures and continuous surveillance of infections among healthcare professionals.

Author Contributions

All authors contributed to the study's conception and design. All authors prepared the materials. AA supervised the whole work. PS collected the data. DK and NA analyzed the data and wrote the first draft of the manuscript, and all authors commented on previous versions. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in connection with the reported study.

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