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ORIGINAL ARTICLE

Alarming Antibiotic Resistance Pattern of Bacterial Isolates in Neonatal Sepsis: A Study from Egypt

[1]Reham M. Raafat*, [1]Reham A. Dwedar, [2]Rasha H. Bassyouni, [3]Ahmed S. Emira, [4]Rehab Galal Abd El-Hmid, [5]Mona A F Dowidar, [1]Asmaa S. Hegab

[1]Medical Microbiology and Immunology Department, Faculty of Medicine, Cairo University, Egypt
[2]Medical Microbiology and Immunology Department, Faculty of Medicine, Fayoum University, Egypt
[3]MD Medical Microbiology and Immunology, Faculty of Medicine, Cairo University, Egypt; Microbiology Department, Alfa Laboratories, Cairo, Egypt
[4]Pediatric Department, Faculty of Medicine, Fayoum University, Egypt
[5]Medical Pharmacology, Faculty of Medicine, Cairo University, Egypt

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Corresponding Author:
Reham Mohammad Raafat Hamed
Lecturer Medical Microbiology and Immunology Department, Faculty of Medicine, Cairo University, Egypt
Tel.: 01020311987
rehamraafat1986@yahoo.com
rehamraafat1986@kasralainy.edu.eg
rehamraafat1986@cu.edu.eg
ORCID: 0000-0002-5526-2134

ABSTRACT

Background: *Sepsis in the neonatal intensive care unit (NICU) remains one of the most significant causes of morbidity and mortality, especially for preterm. Multi-drug-resistant-organisms (MDROs) are emerging as important pathogens that cause neonatal sepsis in NICU.* ***Objective:*** *to review the epidemiology of the microorganisms implicated in neonatal sepsis while shedding the light on the percentage of Multiple-drug-resistant (MDR) and Extensive-Drug-Resistance (XDR) microorganisms in addition to investigating their antibiotic susceptibility pattern.* ***Methodology:*** *This is a cross-sectional prospective study of a 24-month duration including data from culture-proven neonatal sepsis patients admitted at NICU, from Fayoum University Pediatric Hospital.* ***Results:*** *Klebsiella species was the most isolated organism from blood (46%), and 87.6% of isolates were MDR organisms (332/379). The resistance pattern was as follows: 66.5% of resistance owed to Gram-negative bacilli; of them 52% were XDR, 13.2% were MDR, 0.8% were Pan drug Resistant and 0.5% were Difficult-to-Treat (DTR)-Pseudomonas species. Gram-positive cocci were responsible for 21.1 % of MDR; 20.8% MRSA and 0.3% VRE. Total MDR accounted for 34.3% of isolates. The isolates showed significant resistance to most tested antibiotics ($p<0.05$) (doxycycline, tetracycline, amikacin, cefoxitin, meropenem, imipenem, ertapenem, piperacillin-tazobactam, and ampicillin-sulbactam. Significant sensitivity was detected to linezolid, vancomycin, and tigecycline.* ***Conclusion:*** *There is an alarming increase in the resistance rates among cases of neonatal sepsis. The application of an antibiotic stewardship program is essentially needed to stop the dramatic increase in antibiotic resistance and fight the development of MDROs.*

INTRODUCTION

The prevalence of neonatal sepsis (NS) increased in recent times, it may be due to the common use of invasive procedures and the development of resistant organisms. Bacterial resistance to generally used antibiotics has surfaced and complicated the operation of NS. Multidrug-resistant bacteria are defined as acquired non-vulnerability to at least one agent in three or further antimicrobial orders[1].

Sepsis in the neonatal intensive care unit (NICU) remains one of the most common causes of morbidity and mortality, especially in the preterm neonate[2,3]. Neonatal sepsis is divided into early-onset (defined as the onset of sepsis in the first three days of life) and late-onset (after day three of life) sepsis[4]. Early-onset sepsis is generally caused by organisms acquired from the mother's genital tract around the time of delivery (i.e., group B *streptococcus* and enteric Gram-negative bacteria, generally *Escherichia coli* (*E. coli)* and *Klebsiella pneumoniae*) [5]. Late-onset sepsis is generally caused by pathogens acquired during the staying of hospitalization or during delivery, including coagulase-negative *staphylococcus* (CoNS), *Staphylococcus aureus*, enterococcus species, and Enterobacteriaceae[4,6]. Over the last decades, multi-drug resistant organisms (MDROs) have been arising as important pathogens that beget sepsis in the NICU, including extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*[7], methicillin-resistant *S. aureus* (MRSA) [8], and MDR *Acinetobacter baumannii*[9,10].

Infections are considered nosocomial if they first appear 48 h or further after hospital admission or within 30 days after discharge. This type of infection is also known as healthcare-associated infection[11]. Healthcare-

Associated Infections (HAI) constitute an important problem in neonatal units where invasive biases are constantly involved[12]. HAI in the NICU takes numerous forms, and the most frequent forms are bloodstream infections (septicemia) (28%). Bloodstream infection (BSI) is the presence of pathogens in blood culture with clinical signs of infection. Most nosocomial infections are due to BSIs representing up to further than 75% of all HAIs among newborns. Most BSIs are associated with catheter use, in particular central lines like peripherally inserted central catheters (PICC) and umbilical lines. Central line-associated bloodstream infections (CLABSIs) extend hospital length of stay by a normal of 7 days and affect attributable costs. Transmission of MDROs from the hands of the healthcare labor force (HCP) to cases is the primary system of transmission of these organisms in the healthcare surrounding[13].

This study aimed to review the epidemiology of the microorganisms concerned in neonatal sepsis while shedding the light on the percentage of Multi-drug resistant (MDR) and Extensive drug resistance (XDR) microorganisms in addition to investigating their antibiotic susceptibility pattern.

METHODOLOGY

The population of the study:

This study included 1064 cases admitted during the study period (from July 2021 to December, 2022), from them, 379 newborns (35.6%) were diagnosed with sepsis centered on clinical signs and microbiological laboratory results (proven bacteremia and/or clinical sepsis associated with clinical and laboratory values indicating infections).

Study Design:

The present study was a cross-sectional prospective study

Sample size:

This study included 1064 cases, from them, 379 newborns (35.6%) were diagnosed with sepsis (2 blood culture bottles for each patient) for cultivating microorganisms involved in neonatal bacteremia and/or clinical sepsis for neonates including full-term and preterm neonates. Patients were classified into three groups; group I included neonates who were less than 72h, group II included- neonates who were from 72h to 28 d, and group III included neonates from 28d to 60 d.

Inclusion criteria:

The diagnosis of neonates with microbiological bacteremia and/or clinical sepsis was associated with laboratory markers indicating infections. Newborns were diagnosed as having sepsis if they got a score of 3 or more of the following hematologic findings: i) atypical total leucocyte count, ii) abnormal total polymorphonuclear neutrophils (PMN) count, iii) increased immature PMN count, iv) increased immature to total PMN ratio, v) immature to mature PMN ratio ≥ 0.3, vi) platelets count ≤150,000/mm3, and vii) distinct degenerative changes in PMNs[14].

Exclusion criteria:

Excluded patients were neonates with congenital infections and necrotizing enterocolitis along with neonates with intrauterine growth retardation. Also, parents refused to include their babies in the study.

The present study was a cross-sectional prospective study of 24 months duration including prospectively collected data at the neonatal intensive care unit (NICU), in Fayoum University Pediatric Hospital. The study was approved by the faculty of medicine, Fayoum University Ethical Committee under the number (R 179) in its session (84) on 11/7/2021.

Sample collection and processing:

According to the manufacturer's instructions, blood samples for culture were collected aseptically from peripheral veins using butterfly needles (2–3 ml for each blood culture bottle/duplicate samples for each subject) (Bact/Alert 3D, bioMérieux, Durham, N.C., USA). A positive result indicates that there are probably live bacteria in the vial. A five-day strategy was followed for reporting negative blood culture results. Additional subcultures on Chocolate agar, blood agar, and MacConkey's agar for 24 hours at 37^{o}C were carried out once an instrument records a positive signal. The identification of organisms using conventional microbiological techniques required additional processing. For species-level identification of Enterobacteriaceae, the API 20 E identification panel was used. By using Streptex latex agglutination, Group B Streptococci (GBS) were identified in Streptococcus species (Remmel, UK). Based on coagulase testing and growth on DNAse agar, Staphylococcus spp. were identified[15].

Antimicrobial susceptibility test:

Antimicrobial susceptibility of isolates was determined by the standard Kirby Bauer disk diffusion method using antimicrobial discs (Oxoid limited Basingstoke, Hampshire, England) stored according to the manufacturer's instructions. Using Muller Hinton agar (Oxoid, Basingstoke, United Kingdom), all procedures were carried out in accordance with the Clinical & Laboratory Standards Institute (CLSI) guidelines. According to the CLSI recommendations, disc zone sizes were classified as sensitive, resistant, or intermediate based on the breakpoints for disc diffusion tests[15]. Antibiotic sensitivity was assessed for each family of organisms toward the following antibiotics (potency in µg/disc): Cefoxitin (30), Ceftriaxone (30), Ceftazidime (30), Gentamicin (10), Ampicillin (10), Imipenem-Cilastatin (10), Ciprofloxacin (5), Cefotaxime(30), Cefuroxime(30), Tigecycline (15), Cefoperazone-sulbactam(2:1)(105), Trimethoprim-sulfamethoxazole (1:19)(25), Doxycycline (5), Tetracycline (30), Ertapenem (10), Cefepime (30),

Linezolid (30), Colistin (10), Meropenem (10), Cefoperazone (30), Piperacillin-Tazobactam (100/10), Vancomycin (30), Ampicillin-sulbactam (10/10), Amikacin (10), Polymyxin B (300 U), Amoxicillin-clavulanic (20/10) (Oxoid, Basingstoke, United Kingdom).

The cefoxitin (30 µg/disc) disc was used for the phenotypic test for the identification of MRSA. Cefoxitin-susceptible organisms were those with a zone of inhibition equal to or greater than 22 mm, and they were known as methicillin-sensitive Staphylococcus aureus. Methicillin-resistant Staphylococcus aureus isolates were those that exhibited a zone of inhibition that was smaller than or equal to 21 mm (MRSA) [16].

Statistical methods:

Data were entered and coded using SPSS version 28 of the statistical software for the social sciences (IBM Corp., Armonk, NY, USA). Utilizing frequencies (number of cases) and relative frequencies, data were summarised (percentages). Chi square (2) test was used to compare categorical data. Instead, an exact test was utilised when the anticipated frequency was lower than 5. Statistical significance was defined as a P-value equal to or less than 0.05[17].

RESULTS

This study was conducted over 379 isolates. The distribution of isolates by gender and age group is summarized in table 1.

Table 1: Distribution of isolates by gender and age group

		Count	%
Gender	**Male**	239	63.1%
	Female	140	36.9%
Age	**Group (1) less than72h**	75	19.8%
	Group (2) from 72h to 28 d	261	68.9%
	Group (3) from 28d to 60 d	43	11.3%

Sugar fermenter Gram-negative bacilli accounted for 54.1%. Non-fermenter Gram-negative bacilli represented 12.4%, Gram-positive cocci were 28.2% and *Candida* spp. attributed to 5.3%. Five cases were mixed infections (table 2).

Table 2: Types and frequencies of isolated bacteria

Organism type	**Gram-positive**	107	28.2%
	S. aureus	**82**	21.6%
	Enterococci	**22**	5.8%
	Streptococcus pyogenes	**3**	0.8%
	Gram-negative (fermenters)	205	54.1%
	***Klebsiella* spp.**	**174**	46%
	E coli	**18**	4.7%
	***Enterobacter* spp.**	**13**	3.4%
	Gram-negative (non-fermenters)	47	12.4%
	***Pseudomonas* spp.**	18	4.7%
	***Acinetobacter* spp.**	29	7.7%
	***Candida* spp.**	20	5.3%
more than one organism	**Yes**	**5**	1.3%
	No	**374**	98.7%

Regarding resistant pattern of the isolates, 87.6% of isolates were MDROs (332/379); 66.5% of resistance owed to Gram-negative bacilli while Gram-positive cocci were responsible for 21.1 % of MDR. Total MDR accounted for 34.3% of isolates (table 3).

Table 3: Distribution of resistance among isolates

Resistance	**Gram-negative**	**MDR Gram-negative**	50	13.2%
		ESBL	9	2.4%
		Carbapenem Resistant	25	6.6%
		MDR *Pseudomonas* spp.	16	4.2%
		DTR *Pseudomonas* spp.	2	0.5%
		Extensively drug Resistant	197	52%
		Pandrug Resistant	3	0.8%
	Gram-positive	**MDR Gram-positive**	80	21.1%
		MRSA	79	20.8%
		VRE	1	0.3%

The isolates showed higher sensitivities to tigecycline 87.3% and colistin 57.1%. Gram-positive cocci were 99.1% sensitive to linezolid and 98.1% sensitive to vancomycin. 55.2% of isolates were sensitive to more than 2 drugs. 86.1% were sensitive to drugs other than doxycycline and tigecycline.

Bloodstream infections (BSI) were significantly higher in males than females in **Group (1) and Group (2)**; 60% and 67.4% respectively but significantly higher in females than males; 58.1% in **Group (3)** (P value =0.005). Fermenter Gram-negative bacilli showed significantly higher prevalence; 62.1% in **Group (2)** and 40% in **Group (1)** while Gram-positive cocci prevailed at 53.5% in **Group (3)** (*P* value sensitive< 0.001) **(table 4)**.

Table 4: Comparison among age groups

		Age						**P value**
		Group (1)		**Group (2)**		**Group (3)**		
		Count N=75	**%**	**Count N=261**	**%**	**Count N=34**	**%**	
gender	**M**	45	60.0%	176	67.4%	18	41.9%	**0.005***
	F	30	40.0%	85	32.6%	25	58.1%	
more than one organism	**yes**	3	4.0%	2	0.8%	0	0.0%	0.077
	no	72	96.0%	259	99.2%	43	100.0%	
Organism type	**Gram-positive**	22	29.3%	62	23.8%	23	53.5%	**< 0.001***
	Gram-negative (fermenters)	30	40.0%	162	62.1%	13	30.2%	**< 0.001***
	Gram-negative (non-fermenters)	20	26.7%	20	7.7%	7	16.3%	**< 0.001***
	***Candida* spp.**	3	4.0%	17	6.5%	0	0.0%	**< 0.001***

*Statistical significance was defined as a P-value equal to or less than 0.05

The sensitivity pattern to antibiotics among age groups is sumarised in table 5**.** Linezolid, Vancomycin, and Tigecycline were the most active antibiotic among all age groups.

Table 5: Comparison of sensitivity pattern to antibiotics among age groups

		Age						P value
		Group (1) N=75		Group (2) N=261		Group (3) N=34		
		Count	%	Count	%	Count	%	
Tetracyclines								
Doxycycline	**R**	61	84.7%	131	53.7%	37	86.0%	**< 0.001***
	S	11	15.3%	113	46.3%	6	14.0%	
Tetracycline	**R**	66	91.7%	140	57.4%	39	90.7%	**< 0.001***
	S	6	8.3%	104	42.6%	4	9.3%	
Tigecycline	**R**	12	24.0%	16	8.8%	4	20.0%	**0.008***
	S	38	76.0%	166	91.2%	16	80.0%	
Colistin/polymyxin								
Colistin	**R**	31	62.0%	62	34.1%	15	75.0%	**< 0.001***
	S	19	38.0%	120	65.9%	5	25.0%	
Aminoglycosides								
Amikacin	**R**	37	74.0%	153	84.1%	11	55.0%	**0.006***
	S	13	26.0%	29	15.9%	9	45.0%	
β -lactam antibiotics								
Cefoxitin(cephamycin)	**R**	66	91.7%	240	98.4%	41	95.3%	**0.018***
	S	6	8.3%	4	1.6%	2	4.7%	
Ertapenem (cabapenem)	**R**	44	88.0%	179	98.4%	20	100.0%	**0.006***
	S	6	12.0%	3	1.6%	0	0.0%	
Imipenem(cabapenem)	**R**	44	88.0%	178	97.8%	20	100.0%	**0.013***
	S	6	12.0%	4	2.2%	0	0.0%	
Meropenem(cabapenem)	**R**	43	86.0%	179	98.4%	20	100.0%	**0.002***
	S	7	14.0%	3	1.6%	0	0.0%	
Piperacillin-tazobactam(β -lactam/-lactamase inhibitor)	**R**	40	80.0%	175	96.2%	19	95.0%	**0.001***
	S	10	20.0%	7	3.8%	1	5.0%	
Ampicillin-sulbactam (β -lactam/-lactamase inhibitor)	**R**	43	86.0%	179	98.4%	20	100.0%	**0.002***
	S	7	14.0%	3	1.6%	0	0.0%	
	S	11	15.3%	113	46.3%	6	14.0%	
Oxazolidinone								
Linezolid	**R**	0	0.0%	0	0.0%	1	4.3%	0.421
	S	22	100.0%	62	100.0%	22	95.7%	
Glycopeptide								
Vancomycin	**R**	1	4.5%	0	0.0%	1	4.3%	0.175
	S	21	95.5%	62	100.0%	22	95.7%	

*Statistical significance was defined as a P-value equal to or less than 0.05

About 59.8% of **Group (2)** and 53.5% from **Group (3)** showed significant sensitivity to more than 2 drugs (*P* value = 0.013). Isolates sensitive to drugs other than doxycycline and tigecycline accounted for 79.2%, 89.3%, and 79.1% for **Group (1)**, **Group (2)**, and **Group (3)** respectively (*P* value = 0.033) (table 6).

Table 6: Comparison of sensitivity to more than 2 drugs and drugs other than doxycycline (DO) and tigecycline (TGC) among age groups

		Age						P value
		Group (1)		Group (2)		Group (3)		
		Count N=75	%	Count N=261	%	Count N=34	%	
Sensitive to more than 2 drugs	**yes**	29	40.3%	146	59.8%	23	53.5%	**0.013***
	no	43	59.7%	98	40.2%	20	46.5%	
Sensitive to drugs other than doxycycline (DO) and tigecycline (TGC)	**yes**	57	79.2%	218	89.3%	34	79.1%	**0.033***
	no	15	20.8%	26	10.7%	9	20.9%	

* Statistical significance was defined as a P-value equal to or less than 0.05

DISCUSSION

Neonatal sepsis is a worldwide problem that presents a management challenge to care groups for neonates and infants. Early diagnosis and management can considerably decrease the risk of sepsis, and improve the outcome [18,19].

In the present study, Gram-negative sepsis accounted for 66.5% of BSIs. This agreed with previous studies conducted in Egyptian NICUs reporting GNB to represent 62 % and 68% respectively [20, 21]. *Klebsiella* spp. was the most common isolated organism (46%) which is the case in other Egyptian studies'[22,23] conducted by Draz et al[22] (43.3%) and Salama et al[23] (31.8%). The development of multi-drug resistant *K. pneumoniae* (MDRKP) strains leads to a growing global burden in choosing applicable antibiotics in treating hospital-acquired infections[24]. The emergence of MDRKP in healthcare installations could be attributed to the accession of new resistance genes, the use of invasive medical devices, inadequate diagnostic and surveillance systems, immunosuppressed states, and inappropriate use of antibiotics[25]. Unfortunately, the script of antimicrobial resistance has gotten worse, with the appearance of extensively drug-resistant and pan-drug-resistant *K. pneumoniae* strains[26]. Enormous complications generally follow the infection of these" superbugs " and death may be the final case scenario[18,19].

Staphylococcus aureus represents the second cause of neonatal BSIs (21.6%) in the present study. MRSA continues to be a significant source of morbidity for the NICU population. The literal trends of adding the frequency of MRSA in NICUs and elaboration of further MRSA strain types within the sanitarium and community settings suggest that clinical struggles with this pathogen will continue in the future. As the population of NICU cases increases, due to limits of viability being pushed to indeed youngish gravid periods and fleetly advancing technologies enabling further babies to survive, there's an eventuality that numerous further newborns will be at threat for colonization and infection with MRSA[27].

Streptococcus agalactiae played no role in neonatal sepsis in our environment. An Indian study reported that *Streptococcus* spp caused only 2 % of sepsis in NICU. These data differ markedly from those obtained from high-income settings where group B *Streptococcus* is the most common cause of early onset sepsis[28]. *Candida* was isolated in 5.3% of cases in our study. Similar results were obtained in China by Zhang et al[29]. (4.3%). Unexpectedly Draz et al.[22] did not report any invasive candidiasis during their study in an Egyptian NICU. The major contributor to newborn sepsis and mortality from sepsis is Candida bloodstream infection (BSI).[30]. This may be related to the following: premature and very low birth weight (VLBW), central vascular catheterization, parenteral nourishment, use of broad-spectrum antibiotics, H2 blockers, and corticosteroids, endotracheal intubation, and prolonged hospital stay.[30].

We reported 1.3% polymicrobial infections which were greatly lower than the study conducted by Ballot et al [31]in South Africa, who reported 13.8% mixed infections. Polymicrobial BSIs show a two-fold rise in the mortality rate compared to monomicrobial BSI [20].

The results of the antimicrobial susceptibility testing in our NICU are high. XDR, MDR, and PDR accounted for 52%, 34.3%, and 0.8 % respectively. Gupta *et al.* reported a lower prevalence for XDR, higher prevalence for MDR and lower prevalence for PDR isolate in an Indian NICU (33%, 43.5%, and 0 respectively) [32]. In our study, 87.6% of isolates were MDROs. Ain Shams University and Al-Azhar University[33] NICUs reported that 77% of the isolates were multidrug-resistant. In Cairo University Specialized Pediatric Hospital 66.7% were MDRO[34]. MDROs accounted for 85.8% of isolates in neonatal and pediatric intensive care units of Beni-Suef University Hospital[35].

Our isolates showed significantly higher resistance to piperacillin-tazobactam (80.0%, 96.2%, 95.0%; *P* value = 0.001) and ampicillin-sulbactam (86.0%, 98.4%, 100.0%; *P* value = 0.002) for Group (1), Group (2), and Group (3) respectively, compared to an Indian study[29] which reported low and 83.9% resistance rates.

In addition, we discovered noticeably decreased susceptibility to (38.0%, 65.9%, 25.0%; *P* value = < 0.001) among the age groups 1, 2 and3 respectively, than those reported by Salama et al.[23] 96.6% and 100.0% among *Enterobacteriaceae* and non-fermenters respectively and those reported by Negm et al[36]; 96.2%, 94.7%, and 89.9% sensitivity for *Klebsiella* spp., *E. coli, and Acinetobacter*, respectively[37] (86%), so we are losing colistin as an effective therapy. Alarmingly, we are having a paucity of treatment options and getting short to treat infections.

Carbapenems are the medicines of choice for treating infections caused by extended-spectrum beta-lactamase (ESBL)-producing bacteria[38]. Unluckily, there has been an upsurge in carbapenem resistance[39]. Our results revealed a high resistant pattern to meropenem (86.0%, 98.4%, 100.0%; P value =0.002), imipenem (88.0%, 97.8%, 100.0%; *P* value = 0.013), and ertapenem (88.0%, 98.4%, 100.0%, *P* value = 0.006) among the age groups1,2 and 3 respectively.

All *Pseudomonas* spp. isolates were MDROs, accounting for 4.7% of sepsis in our NICU (4.2% MDR *Pseudomonas* spp. and 0.5% DTR *Pseudomonas* spp.) This was the case in other Egyptian studies conducted at Beni-Suef University Hospital which reported that all *Pseudomonas* isolates were MDR, accounting for 8.8% of isolates and at a NICU of a tertiary referral center in Egypt[35] which reported that MDR *Pseudomonas* accounted for 4.5% of isolates. . Due to its virulence and unexpected capacity to acquire additional *in vivo* drug resistance, *Pseudomonas aeruginosa* is one of the most aggressive bacteria, especially in hospital settings and in immunocompromised people. Notably, multiple pathways can frequently exist side by side in a single clinical isolate. Multiple pathways mediate various degrees of resistance to each class of antibiotics, even though they are all tied to a particular class of antibiotics. Multi-drug resistance phenotypes of *P. aeruginosa* isolates have been attributed mostly to OprD deficiency and overproduction of active efflux pumps, AmpC ß-lactamase, extended-spectrum ß-lactamases (ESBL), and carbapenemases, particularly Metallo ß -lactamase (MBL) production. [40]. Depending on the area, each mechanism contributes differently. According to the commonly used definitions, extensively drug-resistant (XDR) *P. aeruginosa* is resistant to all but two or fewer antibiotic classes, including anti-pseudomonal cephalosporins, anti-pseudomonal penicillins plus ß-lactamase inhibitors, monobactams, anti-pseudomonal carbapenems, aminoglycosides, fluoroquinolones, phosphonic acid[1].

CONCLUSION

There is an alarming increase in the resistance rates among cases of neonatal sepsis. The application of an antibiotic stewardship program is essentially needed to stop the dramatic increase in antibiotic resistance and fight the development of MDROs.

Ethical approval: All measures were carried out following the ethical considerations of the faculty of medicine, Fayoum University Ethical Committee under the number (R 179) in its session (84) on 11/7/2021. As well as the ethical standards of the 1964 Declaration of Helsinki. (All parents, babies in the study. provided informed consent prior to data collection and following the explanation of research objectives.

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Authors' contributions: R.M.RM: participated in the construction of the hypothesis,and contributed to writing the manuscript and reviewed the manuscript, made significant revisions to the drafts and corresponding with the journal; R.A.D: performed the techniques and biochemical analysis; A.S.E: performed the techniques and biochemical analysis; R. H.B contributed to reviewed the manuscript, made significant revisions to the drafts; R.G.A: participated in the management of the patient and clinical examinations; M.AF.D **analysis and interpretation of data;** A.S.H: participated in the construction of the hypothesis and contributed to writing the manuscript and reviewed the manuscript, made significant revisions to the drafts

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