Microbes and Infectious Diseases 2022; 3(3): 645-656

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Microbiological profiles of neonatal sepsis in Northern Egypt

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ARTICLE INFO

Article history:
Received 26 March 2022
Received in revised form 24 April 2022
Accepted 24 April 2022

Keywords:
Neonatal sepsis
Early onset sepsis
Late onset sepsis
Multi drug resistance
BACT/ALERT

ABSTRACT

Background: This study aimed at analyzing the microbiological profile of neonatal sepsis in Egypt and to determine the antibiotic susceptibility patterns of the isolated microorganisms. **Methods:** Two thousand and four hundred blood samples were collected from neonates showing symptoms suggestive of septicemia, inoculated into BACT/ALERT culture bottles. Positive blood culture samples were identified and tested for antimicrobial susceptibility. **Results:** Among the neonates included in our study, 457 (19%) neonates were positively diagnosed with sepsis. early onset neonatal sepsis (EOS) was detected in 181 (39.6%), while late-onset neonatal sepsis (LOS) in 276 (60.4%) cases. *Klebsiella pneumoniae* was the most commonly isolated microorganism. *Staphylococcus aureus* and *Enterococcus* spp. were the most common isolated Gram-positive bacteria. *Candida* spp. was more encountered in LOS. An alarming feature of the present study is the high incidence of multidrug resistant microorganisms (65%). Among Gram negative isolates (56%) of were extended spectrum beta-lactamase **(**ESBL) producers and (70.5%) were carbapenem resistant. In Gram positive bacteria, resistance to methicillin in *S. aureus and* coagulase negative *staphylococci* were detected in (50%) and (41%) of isolates respectively. Additionally, 17% of *Enterococcus* isolates were vancomycin resistant. **Coclusion:** Our bacteriological profile of neonatal sepsis showed that Gram negative bacteria represented the majority of isolates. *Klebsiella pneumoniae* was the predominant isolate. In our study, both EOS & LOS share a nosocomial infection profile, as high antimicrobial resistance was observed among our isolates. The susceptibility profiles of the isolates may urge for the change of the current used empirical therapies.

Introduction

Neonatal sepsis is a leading global health problem. The global prevalence is one to ten per one thousand live births. The problem of sepsis is greater in developing countries. The mortality rate of sepsis may reach 50% for untreated newborns. Early onset neonatal sepsis (EOS) is caused by the transmission of pathogens to the fetus from the mother during delivery. Late onset neonatal sepsis (LOS) on the other hand, occurs by transmission of pathogens to the newborn after delivery, such as contact with healthcare workers and Midwives. Neonatal sepsis is manifested with a diversity of systemic signs and symptoms and confirmed by isolation of an infectious agent from the blood [1,2].

Neonatal sepsis is widely classified into EOS and LOS. Early onset sepsis is defined as sepsis within 48-72 hours of birth, it is associated with bacteremia with or without meningitis. The

DOI: 10.21608/MID.2022.129600.1265
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symptoms are characterized by slight early signs which may escalate to fulminant septicemic shock. Transplacental transmission or ascending vaginal infection from the mother are the most common routes of EOS transmission. Late-onset sepsis usually appears three days after delivery. The source of infection in LOS may be nosocomial or community-acquired [3]. Neonatal sepsis caused by multidrug resistant (MDR) microorganisms is commonly associated with even a higher mortality rate. Because of the limited therapeutic options and the high mortality rate, MDR microorganisms associated sepsis is a major problem especially in developing countries [4,5].

The key factor in reducing mortality in neonatal sepsis is the early diagnosis which is crucial for rapid initiation of proper therapy. Blood culture systems have greatly shortened detection time and increased sensitivity [6].

Reducing mortality and morbidity among the neonates with sepsis requires more effective diagnosis and continuous monitoring of the efficacy of the treatment used. To achieve this, we need a much better understanding of pathogens prevalence, and their antimicrobial susceptibilities The present work was carried out as a cross sectional study to analyze the microbiological profile of neonatal sepsis in different hospitals in Alexandria and El Behira (northern Egypt), to determine the antimicrobial susceptibility profile of the isolated microorganisms, and to detect the major antibiotic resistance patterns.

Materials and Methods

This study was carried in the period from February 2018 until February 2019. Blood culture samples were collected from three main referral hospitals: El Shatby University Hospital (Alexandria), Mabart al Asafra Hospital (Alexandria), and Damanhur General Hospital (EL Behira). An informed consent was taken from the infant parent before sampling. The study was approved from ethics committee of medical research institute, Alexandria University, Approval No. E\C. S\N. T2\2017.

Sample collection

Two thousand and four hundred blood samples were collected randomly from all neonates showing any of the following symptoms of septicemia such as hypothermia or fever, lethargy, absent neonatal reflexes, brady/tachycardia, respiratory distress, apnea or gasping respiration and/or hypo/hyperglycemia [7].

Blood samples were withdrawn for culture and immediately inoculated at 37 °C in the BACT/ ALERT pediatric culture bottles in the hospital laboratory. Bottles were incubated in BacT/ALERT 3D automated culture system within 1hour of the collection and were monitored for growth by detection of fluorescence changes.

Isolates identification and susceptibility testing

BACT/ALERT bottles that gave positive alarm were further analyzed in medical research institute microbiology lab. The positive bottles were sub cultured on blood agar, MacConkey's agar, chocolate agar and Sabouraud's dextrose agar (SDA) plates and incubated at 37°C for 24-48 hours. The identification of isolated microorganism as well as susceptibility testing were performed using VITEK 2 (bioMerieux, Inc., France) compact system according to manufacture instructions. Gram positive (GP) ID, Gram negative (GN) ID and Yeast ID cards were used for identification of Gram-positive bacteria, Gram-negative bacteria and yeast isolates respectively. While, GP AST 592, GN AST 71, GN AST 222 and Yeast 008 cards were used for antimicrobial susceptibility testing of Gram-positive, Gram-negative fermenter, Gram-negative non-fermenter and *Candida* isolates respectively. The minimum inhibitory concentration (MIC) of antibiotics were interpreted according to Clinical and laboratory standards institute (CLSI) guidelines 2019 to sensitive, intermediate, and resistant strains [8].

Phenotypic detection of antibiotic resistance mechanism

BioMérieux chrom ID agars were used for Phenotypic resistance pattern detection according to manufacturer instructions. For Gram-positive bacteria MRSA Chrom ID and VRE ChromID agars were used for detection of methicillin resistant *Staphylococcus aureus* and vancomycin resistant *enterococci* (VRE) respectively.

For Gram-negative bacteria, ESBL ChromID and CARBA Chrom ID agars were used for detection of ESBL and carbapenemase producing Enterobacteriaceae.

Data were analyzed using SPSS ver.20 Chicago, IL, USA, , Pearson Chi square test was used, the level of significance was 0.05, below which the results are considered to be statistically significant.

Results. Two thousand four hundred neonates with suspected septicemia were admitted during the study period. Sepsis was confirmed only in 457 (19%) neonates with positive blood cultures. EOS was presented in 181 (39.6%) cases, while LOS was presented in 276 (60.4%). Most of EOS cases 114/181 (63%) were males, while the majority of LOS 170/276 (61.5%) were females **(Table 1)**.

Generally, Gram-negative bacteria 336/457(73.6%) were more frequently isolated in both EOS and LOS, followed by Gram positive bacteria 90/457 (19.6%), and fungi 31/457 (6.8%), the rate of fungal isolation was significantly higher among LOS cases.

Klebsiella pneumonia was the most frequently isolated Gram-negative bacteria 245/336 (73%) followed by *Escherichia coli* 34/336 (10%), and *Acinetobacter baumannii* 22/336 (6.5%). *Pseudomonas aeruginosa* 2/457 (1.5%) was exclusively isolated from LOS. Other low prevalence 15/336 (4.5%) Gram-negative bacteria were isolated. These included *Brevundimonas diminuta, Pseudomonas auryzihabitans , Pantoea species, Burkholderia capicia, Achromobacter xylosoxidans, Pandoraea* spp. *Cupriavidulus pauculus, Achrombacter dinitrificans , Aeromonas hydrophila, Aeromonas sopria*, and *Burkholderia gladioli* **(Table 2)**.

Table 3 and **4** show the antimicrobial susceptibility pattern for the isolated microorganisms, performed using different antimicrobial agents suggested by VITEK2 system.

Regarding Gram-positive bacteria, *S. aureus* was the most frequently isolated strain (30/90, 33%). The rate of *S. aureus* detection among EOS cases (60%) was found to be significantly higher compared to LOS (40%). *Enterococcus faecalis* and coagulase negative Staphylococci were the second most isolated Gram-positive bacteria 17/90 (19%) each. The remaining Gram-positive isolates were divided between *Enterococci* Other than *faecalis*, and *Streptococcus spp*. 13/90(14%) each. Both isolates were significantly higher among LOS cases.

Regarding fungal isolates, non-albicans *Candida* species were only isolated from LOS cases and accounted for 23/31 (74%) of isolates with a predominance of *C. parapsilosis* 12/31(39%), followed by, *C. tropicalis* 7/31 (23%), *C. glabrata*, and *C. spherica* 2/31 (6%). While *C. albicans* was isolated in 8 /31(26%) cases.

Concerning *K. pneumoniae*, the most frequently isolated Gram-negative bacteria, resistance to penicillin's/β-lactamase inhibitors, extended spectrum cephalosporins, monobactams and Carbapenems ranged from 84%-100%. Resistance to Tobramycin, Gentamicin and Amikacin was detected in 86, 72 and 63% of isolates respectively. Resistance to fluoroquinolones and co-trimoxazole was detected in 70% and 76% of isolates respectively. The most effective antibiotics against *K. pneumoniae* were Colistin and Tigecycline. Nevertheless, resistance to these antibiotics was observed in 5% and 1% of isolates respectively.

Generally, colistin and tigecycline resistance were the least encountered among all Gram-negative isolates. However, 18 and 4 isolates showed resistance to them respectively. Noteworthy, 2 out 8 (25%) of *Stenotrophomonas* isolates were resistant to co-trimoxazole and Ceftazidime, which are the drug of choice for this microorganism.

In this study, β-lactam resistance mechanism was tested using chromID agar which showed that 189/336 (56%) Gram negative isolates were ESBL producers and 237/ 336(70.5%) were Carbapenamase producers. There is no significant difference in the distribution of ESBL and carbapenamase production among EOS and LOS cases **(Table 5)**.

Concerning *S. aureus*, the most frequently isolated Gram-positive bacteria, resistance to Ampicillin was detected in 83% of isolates. Half of isolates were resistant to Cefoxitin and diagnosed as Methicillin resistant *S. aureus* (MRSA). Resistance to Gentamicin and co-trimoxazole was observed in 47% of isolates for each. Resistance to fluroquinolones, erythromycin and clindamycin ranged from 7-23%. All *S. aureus* isolates were susceptible to vancomycin, teichoplanin and linezolid.

Methicillin resistance was detected in 50% and 41% of *S. aureus* and coagulase negative *staphylococci* (*CoNS*) respectively. On the other hand, Vancomycin resistance in Enterococci was observed in 17% of isolates. Both Methicillin resistance and Vancomycin resistance were significantly higher among LOS cases table 5.

Collectively, 297 out of 457 isolates (65%) were MDR that showed resistance to one or more agent in more than 2 families of antimicrobial agents. On the other hand, extensively drug-

resistant bacteria (XDR) were observed in 19 out of 457 isolates (4%) that showed resistance to all classes of antimicrobial agents except one. XDR was significantly higher among LOS cases. No pan drug-resistant bacteria (PDR) were observed among our isolates.

In the current study, most *Candida* spp. isolates were sensitive to all tested antifungals, however, 6 isolates were resistant to fluconazole (one isolate of *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. spherica*, and two isolates of *C. albicans*). Additionally, only one *C. spherica* isolate was resistant to voriconazole.

Table 1. Demographic data and sample description.

	EOS 181 (39.6%)	LOS 276 (60.4%)	Total 457 (100%)
Male	114 (52%)	106 (48%)	220 (48%)
Female	67 (28%)	170 (72%)	237 (52%)
Gram Positive	39 (43%)	51 (57%)	90 (19.6%)
Gram Negative	137(41%)	199 (59%)	336 (73.6%)
Fungi*	5 (16%)	26(84%)	31 (6.8%)

* Significant, *p* value <0.05: significant higher isolation among LOS cases

Table 2. Prevalence of microorganisms isolated from positive blood cultures of neonates with early and late onset sepsis.

		EOS	**LOS**	**Total (457)**
Gram negative	*Klebsiella pneumoniae**	116 (47%)	129 (53%)	245 (54%)
	Escherichia coli	10 (29%)	24 (71%)	34 (7%)
	Acinetobacter baumannii complex	6 (27%)	16 (73%)	22 (5%)
	*Pseudomonas aeruginosa**	0	7 (100%)	7 (1.5%)
	*Klebsiella pneumonia sub.*spp. *oxytoca*	1 (20%)	4 (80%)	5 (1%)
	Stenotrophomonas maltophilia	2 (25%)	6 (75%)	8 (2%)
	Other Gram-negative bacteria***	2 (13%)	13 (87%)	15 (3%)
Gram positive	*Staphylococcus aureus***	18 (60%)	12 (40%)	30 (7%)
	Enterococcus faecalis	9 (53%)	8 (47%)	17 (4%)
	*Enterococcus faecium**	0	13 (100%)	13 (3%)
	Coagulase Negative Staphylococci	9 (53%)	8 (47%)	17 (4%)
	Streptococcus spp.*	1 (8%)	12 (92%)	13 (3%)
Fungi	*Candida albicans*	5 (62.5%)	3 (37.5%)	8 (1.5%)
	*Candida parapsilosis**	0	12 (100%)	12 (3%)
	*Candida tropicalis**	**0**	**7 (100%)**	**7 (1.5%)**
	Candida glabrata	**0**	**2 (100%)**	**2 (0.4%)**
	Candida sherica	**0**	**2 (100%)**	**2 (0.4%)**

* *p* value <0.05: significant higher isolation among LOS cases

** *p* value <0.05: significant higher isolation among EOS cases

*** *Brevundimonas diminuta, Pseudomonas auryzihabitans , Pantoea species, Burkholderia capicia, Achromobacter xylosoxidans, Pandoraea* spp. *Cupriavidulus pauculus, Achrombacter dinitrificans , Aeromonas hydrophila, Aeromonas sopria*, and *Burkholderia gladioli.*

Table 3. Antimicrobial susceptibility and resistance pattern of Gram negative isolates.

	% Resistance in Gram negative isolates							
	Klebsiella pneumonia n=245	*Klebsiella oxytoca n=5*	*E. coli n=34*	*Acinetobacter baumannii N=22*	*Pseudomunas aeruginosa n=7*	*Stenotrophomonas maltophilia n=8*	*Others* N=15*	Total (336)
Amikacin	154 (63%)	2 (40%)	7 (20%)	15 (69%)	2 (29%)		5(34%)	185(55%)
Amoxicillin / Clavulanic acid	245 100%	3 (60%)	23 (67%)				14(94%)	285 (85%)
Ampicillin / Sulbactam	245 (100%)	0	20 (58%)	18 (82%)			12(80%)	295 (88%)
Azetrionam	218 (89%)	0	15 (44%)	19 (88%)	7 (100%)		10(60%)	269(80%)
Cefpime	238 (97%)	0	15 (48%)	15 (69%)	5 (71%)		5(34%)	278(83%)
Cefoperazone	218 (98%)	0	20 (58%)	22 (100%)	7 (100%)		12(80%)	279(83%)
Ceftazidime	235 (96%)	0	18(53%)	15 (69%)	5 (71%)	2 (25%)	6 (40%)	281(84%)
Ceftriaxone	218 (86%)	1 (20%)	18(53%)	21 (94%)	7 (100%)		10(67%)	275(82%)
Ciprofloxacin	174 (69%)	2 (40%)	12(35%)	14 (63%)	5 (71%)		6 (40%)	213(63%)
Colistin	12 (5%)	0	0	0	0		6 (40%)	18 (5%)
Gentamicin	176 (72%)	0	10 (29%)	14 (63%)	5 (71%)		7 (47%)	212(63%)
Imipenem	206 (84%)	0	15 (44%)	15 (69%)	5 (71%)		5(34%)	247(74%)
Levofloxacin	174 (71%)	0	10 (29%)	15 (69%)	7 (100%)	0	10(67%)	216(64%)
Meropenem	211 (86%)	0	7 (20%)	15 (69%)	5 (71%)		4 (27%)	242(72%)
Tigecycline	3 (1%)	0	0	0		0	1(7%)	4((1%)
Tobramycin	211(86%)	0	10 (29%)	14 (63%)	5 (71%)		10(60%)	250(74%)
Co-trimoxazole	186 (76%)	2 (40%)	18(53%)	14 (63%)	7 (100%)	2 (25%)	4 (27%)	233(69%)
resistance mechanism								
ESBL	163(66.5)	0	26 (76.4%)	0	0	0	0	189(56%)
Carbapenem resistance	210(85.7)	0	10 (29.4%)	12 (54%)	3 (42.9%)	2 (25%)	0	237(70.5%)

* *Brevundimonas diminuta, Pseudomonas auryzihabitans , Pantoea species, Burkholderia capicia, Achromobacter xylosoxidans, Pandoraea* spp. *Cupriavidulus pauculus, Achrombacter dinitrificans , Aeromonas hydrophila, Aeromonas sopria*, and *Burkholderia gladioli.*

Table 4. Antimicrobial susceptibility and resistance pattern of Gram positive isolates

% Resistance in Gram positive isolates					
	Streptococcus spp. *n=13*	*Staphylococcus aureus n=30*	*CONs N=17*	*Enterococcus* Spp. *n=30*	Total (90)
Ampicillin	1 (8%)	25 (83%)	17(100%)	3 (10%)	46 (51%)
Cefoxitin	0	15 (50%)	7(41%)	30 (100%)	52(58%)
Ciprofloxacin	2 (15%)	4 (13%)	6 (35%)	7 (23%)	19(21%)
Clindamycin	5 (38%)	5 (17%)	0	27 (90%)	37(41%)
Erythromycin	3 (23%)	7 (23%)	11(65%)		21 (23%)
Gentamicin	13 (100%)	14 (47%)	0	27 (90%)	54(60%)
Imipenem	0	15 (50%)	17(100%)	3 (10%)	35 (39%)
Linezolid	0	0	0	0	
Moxifloxacin	3 (23%)	2 (7%)	6 (35%)	6 (20%)	17(19%)
Teicoplanin	0	0	0	5 (17%)	5 (5.5%)
Co-trimoxazole	12 (92%)	14 (47%)	11(65%)		27(30%)
Vancomycin	0	0	0	5 (17%)	5(5.5%)
Resistance mechanisms					
Methicillin resistance		15 (50%)	7 (41%)		22(24%)
Vancomycin resistance				5 (17%)	5(5.5%)

Table 5. Comparison between the isolated resistant strains patterns and resistance mechanisms in EOS & LOS.

Resistance strain	EOS (181)	LOS (276)	Total (457)
Resistance patterns			
MDR	127 (43%)	170(57%)	297 (65%)
XDR*	4 (21%)	15 (79%)	19 (4%)
Resistance mechanisms			
ESBL	89 (47%)	100 (53%)	189 (41%)
Carbapenem resistance	98 (41%)	139 (59%)	237 (52%)
VRE*	0	5 (100%)	5 (1%)
Methicillin resistant CONs*	0	7 (100%)	7 (1.5%)
MRSA*	6 (40%)	9 (60%)	15 (3%)

* Significant, *p* value <0.05: significant higher isolation among LOS cases.

Discussion

Our results showed predominance of Gram-negative bacteria (73.6%) followed by Gram-positive bacteria (19.6%) and fungi (6.8%). Predominance of Gram-negative septicemia was also observed in other geographical areas in Egypt. In a study carried out in neonatal intensive care unit NICU at Cairo University hospital, Gram-negative bacteria were more frequently isolated from neonates [9]. Also, in a 3.5-year retrospective study carried out in Ain Shams University. Gram-negative was found to be the leading cause followed by Gram-positive bacteria and fungi [10].

However, different results were reported by a study carried in three NICUs at Mansoura University Children Hospital, Egypt, where Gram-positive bacteria were responsible for most cases of neonatal sepsis [11]. This could be explained by the time in which the study was conducted, as the sampling time reported was in 2011. In Africa, an Ethiopian study [12] showed that among 120 cases of neonatal sepsis, Gram-positive bacterial species were more commonly isolated (67.5%) than the Gram negative bacterial species (32.5%) however, in this study the sample size reported was small.

Klebsiella pneumoniae was the most frequently isolated Gram-negative bacteria followed by *E. coli*. Similarly, in a study conducted in Beni Suef, Egypt, *K. pneumoniae* was the most common pathogen; accounting for 59 out of 138 isolates (42.8%). [13]. Also, in Tanta University Hospital, Egypt. Among a total of 145 cases, the most common microorganism causing sepsis was *K. pneumoniae* (22.89%) [14]. Moreover, in an Indian study [15] stated that *K. pneumoniae* (45.61 %) was the most common isolated pathogen. The high prevalence of *K. pneumonia* in both LOS and EOS may indicate a common source of infection which could be hospital acquired.

In our study, the rate of *S. aureus* detection was found to be significantly higher among EOS cases (60%). *Enterocoocus faecalis and CoNS* were the second most isolated Gram-positive bacteria. In a Pakistani study [16], they showed that the most common Gram-positive bacteria were *S. aureus* (64.1%), followed by *Enterococcus faecalis* (13.9%). Similarly, a Chinese study [17] stated that out of the 64 culture-positive cases, *S.aureus* was the most frequently isolated microorganism 24 (37.5%). Also in Africa, the previously mentioned Ethiopian study [12] showed that the commonly isolated Gram-positive bacteria were *S. aureus* 49 (40.9%) followed by *CoNS* 26 (21.7%). On the other hand, the aforementioned study conducted in Mansoura University children hospital [11], Gram-positive bacteria were responsible for most cases of neonatal sepsis, and *CoNS* were the most frequent isolated pathogens in both EOS and LOS.

In the current study, *Enterococcus* spp. accounted for 33% of Gram-positive bacteria. *Enterococcus faecalis* accounted for the majority of *Enterococcus* isolates (17/30) and were almost equally distributed between EOS and LOS. on the other hand, *E. faecium* was solely isolated from LOS cases. Our finding was also endorsed by another study [18] on *Enterococcus* spp. among newborn, using PCR they detected. *Enterococcus faecalis* in 22 cases, while *E. faecium* in 8 out of the 40 cases included in their study.

In the current study, *Candida* spp. was less frequently isolated than bacterial pathogens 31/457 (6.8%). Similar findings were reported by in another study [19], they reported the isolation of yeasts in 5.3% of cases of neonatal sepsis. However, a higher incidence was reported in India, a study [20] reported isolation of 32 (9.9%) *Candida* isolates out of 322 positive cases. In our study *Candida* spp. was mostly isolated from late onset sepsis (26/31). Non albicans *Candida* represented the majority of our fungal isolated (23/31). Several factors explain the incidence of candidiasis in LOS such as prolonged endotracheal intubation, central vascular catheters, parenteral nutrition, and the use of broad-spectrum antibiotics. It has been reported *Candida* associated sepsis is linked to extensive use of cefotaxime as an initial empirical antimicrobial in early-onset neonatal sepsis [21,22]. This could be considered as secondary infection as a result of hospitalization.

In our study the Gram-negative isolates showed low level of resistance against colistin (5%) and tigecycline (1%). In accordance, a previous study also observed tigecycline low resistance level (less than 5%) among the neonatal sepsis isolates [23]. Another retrospective study noticed that among 28 different Gram-negative isolates only one was resistant to colistin. Previously, colistin was used infrequently, largely due to the associated nephrotoxicity and neurotoxicity; however, the incidence of these adverse events does appear to be lower with modern preparations. In our study, colistin resistance was encountered among *K. pneumoniae* isolates in 12/254 (5%). These results were consistent with an Egyptian study conducted on 139 carbapenem resistant *K. pneumoniae* (CRKP), in which neonatal sepsis represented 54% of the cases. It was found that 13.8% of CRKP isolated from neonates, were resistant to colistin [24]. **Bialvaei and Samadi** stated that increasing use of colistin for the treatment of infections caused by these bacteria has led to the emergence of colistin resistance in several countries worldwide. Although resistance to polymyxins is generally less than 10%, it is higher in the Mediterranean and Asia (Korea and Singapore), where colistin resistance rates are continually increasing [25]. This could be explained by the misuse of these antibiotics in these areas.

Concerning *K. pneumoniae*, susceptibility profile in our study, was similar to that reported in an Indian study, [26] reported high resistance rates among *K. Pneumonia* isolates, 97% were resistant to ceftazidime and ceftriaxone, 100% to gentamycin, and 82% to ciprofloxacin. However, no carbapenem resistance was demonstrated. on the other hand, an Ethiopian study [27] showed lower resistance rates, where 84.2% of *K. pneumoniae*

isolates were sensitive to ciprofloxacin and gentamycin. It is worth to mention that a Jordanian study [28] which reported nearly the same pattern of microorganisms' distribution but a different susceptibility antibiotic profile. *K. pneumoniae* isolates showed no resistance to Imipenem, 50% to ceftazidime and 8.5% to ciprofloxacin. this could be explained by their national antibiotic restriction policy.

In our study, no significant difference between EOS & LOS regarding ESBL and carbapenamase producing isolates. *Klebsiella pneumoniae* was the most common carbapenamse producing bacteria (85.7%) while *E. coli* was the highest ESBL producing bacteria *(*76.4%). However, a lower rate of carbapenamase producing *K. pneumoniae* isolation was reported in a study on extensively resistant *K. pneumoniae* conducted in El Minia, Egypt. They reported only 24 (15.3%) carbapenem resistant *K. pneumoniae* isolates among their 156 sepsis diagnosed neonates [29].

In accordance with our study, a study carried out in Nepal [30] mentioned that more than half of the total isolates were ESBL producing. Also, in India a study [31] in which Gram-Negative rods were screened and tested for ESBL production. A total of 119 were tested for the harboring extended-spectrum β-lactamase enzyme. The Majority of Klebsiella (55.3%) were ESBL producing. Also, the previously mentioned south African study [32] reported that 65% of the *K. pneumoniae* isolates were ESBL producers.

On the other hand, an Iranian study [33] demonstrated a lower ESBL isolation rate among their *Klebsiella* and *E. coli* isolates (44% and 43.7% respectively). Similarly, another study [34] stated that 62.5% of the *Klebsiella* isolates and 20% of *E. coli* isolates were ESBL producing.

Carbapenemase and ESBL-producing *Enterobacteriaceae* have emerged as an important global health issue, accompanied by wide dissemination and high mortality rates. The indiscriminate use of antibiotics as empirical therapy to treat the multidrug-resistant pathogens might be responsible for the emergence of these resistant strains especially in the hospital settings [35].

In our study *S. aureus* showed high resistance against β lactams , intermediate sensitivity to Aminoglycosides and co-trimoxazole, and low resistance rate among macrolides, while no resistance was detected to vancomycin, teicoplanin and linezolid. On the other hand, In Nigeria a study [36]. showed that, β lactams had moderate to high activity, except for of cloxacillin, which showed no activity against S. *aureus* isolates. Gentamicin had activity similar to that of β lactams. Erythromycin, chloramphenicol, tetracycline, and co-trimoxazole showed no activity against the bacterial isolates.

In our study methicillin resistance was detected in nearly half of *S. aureus* and *CoNS* isolates. While vancomycin resistance in *enterococci* was observed in 17% of isolates.

This was similar to the aforementioned study [30] that showed that (41.1%) *Staphylococci* were MRSA. In an Ethiopian study [12], they found that among the total isolates of *S. aureus*, 13 (26.5%) were methicillin-resistant.

Regarding *Candida* spp. sensitivity, non-*albicans* isolates showed lower resistance rate to Fluconazole 4/23 (17%) than *C. albicans* 2/8 (25%). This was consistent with a study [20] , in which they showed that nonalbicans *Candia* were more susceptible to fluconazole (57% *C. albicans*)

Multidrug resistant sepsis leads to higher mortality and morbidity among neonates. Risk factors for spread of MDR may include irrational antibiotics use, poor sanitation, lack of an effective antibiotic stewardship [37]. Our study showed high incidence of MDR (65%), with no statistical significance difference between LOS EOS. XDR isolates were significantly higher in LOS 15/276(5%) than EOS 4/181 (2.2%) Similar isolation findings were reported in 2016 in a study conducted in Alazhar university, Cairo [10]. They reported that among their isolates 77% were MDR.

Our finding may point out that neonatal sepsis in Egypt, either EOS or LOS could be considered merely a hospital acquired infection. This can be supported by the fact that both EOS and LOS infections share a MDR resistance profile, high incidence of ESBL and carbapenem resistance. In every neonatal sepsis case, there is a greater possibility to be an infection with a resistant strain.

Rapid intervention and empiric treatment is recommended for neonates showing any signs of neonatal sepsis. Penicillin and gentamicin combination therapy is recommended as first-line treatment for both EOS and LOS. Third-generation cephalosporins, such as ceftriaxone, are

recommended as second-line treatment. And carbapenems as the third line of treatment [38].

In the light of the susceptibility results in our study, the antibiotics used in the first and second guidelines of treatment showed intermediate susceptibility among gram positive isolates (50%-60%) and a much lower resistance rate to the third line (39%). While among Gram negative isolates the studied antibiotics showed a higher level of resistance (63%-85%). Similarly in a study evaluated the antibiotic regimens used in Asian countries, meropenem had the highest susceptibility [39]. On the other hand, in a study aimed at assessing the empirical treatment in England, they reported that the guidelines for empirical therapy in neonates with sepsis are appropriate specially gentamicin-based combination regimens [40]. Combination therapies (such as used in the first line) may provide an effective regimen for strains showing intermediate level of resistance, however the susceptibility profiles presented in our study may suggest the urgency to shift the used regimens to carbapenem based one.

Conclusion

Our bacteriological profile of neonatal sepsis showed that Gram negative bacteria represented the majority of isolates. *K. pneumoniae* was the predominant isolate. Neonatal sepsis in Egypt, both EOS and LOS share a nosocomial infection profile, high antimicrobial resistance was observed among our isolates. In every neonatal sepsis case, there are a greater possibility to be an infection with a resistant strain. The susceptibility profiles of the isolates may urge for the change of the current used empirical therapies.

Conflict of interest

No conflict of interest.

Funding: None.

Ethics approval

Approval of this study was obtained from the Ethical committee at Medical research institute, Alexandria university. The study was conducted accordance with the standards set forth in the Declaration of Helsinki principles of 1975, as revised in 2013 (http://ethics.iit.edu/ecodes/node/3931)

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