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Original paper

A six-year evaluation of sepsis in neonates

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Abstract

This study was undertaken to describe and compare the clinical presentations, bacteriological profiles and antibiotic sensitivity patterns of isolates from blood cultures of neonates admitted in Neonatal Intensive Care Unit at Emergency County Hospital Oradea for six years.

All blood culture from neonates with clinical signs of septicaemia (n=485) were collected and analysed in the clinical and laboratory context. The positive blood culture rate found was 7.83%. We considered two groups: clinical and laboratory proven sepsis (at least one positive culture) and unproven sepsis (bacteriological negative, sepsis screen negative but clinical course compatible with sepsis).

Blood culture positivity was found in 7.83%. In both groups (proven and unproven) predominates the early onset of sepsis. Preterm and small for gestational age babies were more affected by septicaemia. The most prevalent clinical feature observed were respiratory syndrome and temperature dysregulation, followed by difficulties to feeding and jaundice. Significant laboratory findings in this study included leukopenia, score system more than 4 and increased CRP. In our group, more than one half of identified microorganisms were gram-positive. The most common gram-negative microorganism isolated was *E. coli*. Our study has shown lower antibiotic resistance compared to the rates reported in the literature.

Early onset was more frequent than late onset sepsis and mortality was relatively higher. The common predisposing neonatal factors for early onset sepsis are prematurity, low birth weight and maternal risk factors. Despite efforts to diagnose and manage sepsis, this condition remains an important public health issue.

Keywords Antibiotics, haematological score, neonatal sepsis.

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Introduction

Systemic infections represent one of the most significant diseases that cause neonatal mortality worldwide, most of them appearing in neonatal intensive care units and occurring especially in premature infants. More than 40% of deaths that appear in under five-year-old population worldwide occur in the neonatal period, resulting in 3.1 million new-born deaths each year (UNICEF [1]). Most of these deaths usually occur in low-income countries and almost 1 million of these deaths are attributed to infectious causes like neonatal sepsis, meningitis, and pneumonia (BLACK & al [2]).

In Romania the infant mortality indicator continues to be the highest in Europe, despite numerous measures taken in this direction (DAINA & al [3]. Worldwide, sepsis is considered an important public health issue, a challenge both of diagnosis (laboratory analysis, imaging and invasive techniques) and clinical management (antibiotherapy), prevention playing an important role in patient safety (DAINA & al [4]).

Neonatal septicaemia is defined as a bacterial infection documented by a positive blood culture in the first four weeks of life. The highest rates of neonatal septicaemia occur in low birth weight infants, those with depressed status at birth as manifested by a low Apgar score, those with maternal perinatal risk factors (e.g.: low socioeconomic status, premature rupture of membranes, maternal chorioamnionitis, colonization with group B streptococcus and minorities).

Neonatal sepsis may be categorized as early-onset sepsis (EOS) or late-onset sepsis (LOS). Early-onset sepsis (present within 24-72 hours after birth) usually results from organisms acquired intrapartum (*Group B streptococcus* and gram-negative enteric organisms, predominantly *Escherichia coli*) for most cases. The implementation of prenatal screening and treatment for group B streptococcus was followed by a decreasing of the incidence of this disease. *Coagulase-negative staphylococcus*, *Haemophilus influenza*, *Listeria monocytogenes* have also been identified in neonates, especially premature neonates.

Late-onset sepsis (in the first 4-90 days of life) is usually acquired from the environment (hospital-acquired neonatal infection). *Staphylococci* account for 30 to 60% of late-onset cases and infections appears due to intravascular devices like central vascular catheters. *E. coli* is also becoming increasingly recognized as a significant cause of late-onset sepsis, especially in extremely low birth weight infants. They are more susceptible to infection caused by common organisms such as *Coagulase-negative staphylococcus*, an organism usually not associated with severe sepsis. Contaminated respiratory equipment is suspected in outbreaks of hospital-acquired *Pseudomonas aeruginosa* pneumonia or sepsis. Trends in late-onset sepsis showed an increase in *Coagulase-negative staphylococcus* sepsis and most of these isolates are susceptible to a first-generation cephalosporin (KLINGER & al [5]; VAN DEN HOOGEN & al [6]). *Candida species* are becoming increasingly important causes of late-onset sepsis, occurring in 12 to 18% of extremely low birth weight infants. Late-onset sepsis is associated with the following risk factors: prematurity, central venous catheterization (duration >10 days), nasal cannula or continuous positive airway pressure use, H2-receptor blocker or proton pump inhibitor use, gastrointestinal tract pathology (GRAHAM & al [7]).

The early diagnosis of sepsis and stratification of its severity is necessary to prevent deaths and complications. Clinical signs are nonspecific and laboratory diagnosis is time consuming. Blood culture remains the gold standard for definitive diagnosis of septicaemia, but the results are time consuming. Biomarkers can have an important place in this process because they can indicate the presence or absence or severity of sepsis, and it can differentiate bacterial from viral and fungal infection, and systemic sepsis from local infection (ZAMBON & al [8]; DOWNING & al [9]; MARSHALL & al [10]).

The aim of the present study was to evaluate clinical and laboratory tools for diagnosis of neonatal sepsis, to characterize the microbiological pattern of neonatal sepsis, the antibiotic susceptibility of the isolates and survival rates.

Materials and Methods

This descriptive retrospective study was performed between January 2011 and December 2017 at Emergency County Hospital Oradea, Romania. The medical records of patients, both mothers and neonates, enrolled in our study were examined and those who had inadequate medical and microbiological data were excluded from the study. Data of the neonates and mothers: maternal risk factors, gestational age, diagnoses, gender, birth weight, mode of delivery, interventional procedures, species of the isolated microorganisms and antibiotic sensitivity were noted and evaluated.

Neonates were enrolled based on the presence of signs and symptoms of clinical sepsis after a complete clinical examination and calculation resulting the sepsis score. The clinical criteria considered were: poor appetite, irritability/excessive crying, lethargy, poor crying and reflexes, fever, hypothermia, jaundice, vomiting, abdominal distension, tachypnea and grunting, convulsions, diarrhoea, pustules, cyanosis, bulged fontanelle, bleeding, poor perfusion/shock, apnoea. Also, significant predisposing factors for presumed early onset sepsis were taken into consideration during inclusion of cases. Exclusion criteria were: congenital anomalies (of gastro-intestinal system, respiratory system, and the cardiovascular system), inborn errors of metabolism, and congenital anomalies of central nervous system.

The early onset sepsis was considered if it appeared below 72 hours of age and late onset sepsis if it appeared after 72 hours of age.

Hematologic findings and complete blood cell count criteria were evaluated as screening tests for neonatal sepsis using automated haematology analyser and peripheral blood smears. From the data obtained, a hematologic scoring system was formulated that assigns a score of 1 for each of seven findings:

• abnormal total leukocyte count: leukopenia < 5000 cells/mm^3 or leucocytosis ≥ 25000/mm^3 at birth; ≥ 30000/mm^3 at 12-24h; ≥21000/mm^3 at 2 days;

• abnormal total neutrophil (PMN) count: under 1800 cells/mm^3 or more than 5400/mm^3;

• elevated immature PMN count;

• elevated immature to total PMN ratio > 0.2;

• immature to mature PMN ratio greater than or equal to 0.3;

• platelet count less than or equal to 100,000/mm^3;

• pronounced degenerative changes in PMNs: toxic granules/cytoplasmic vacuoles.

Monroe devised a score which used these parameters, total PMN count, immature PMN count and I: T ratio (MANROE & al [11]). The higher the score was the greater the likelihood of sepsis. With a score less than or equal to 2 the likelihood that sepsis was absent was 99%. Infants with sepsis and probable infection had scores greater than or equal to 3 or 4, compared with infants without infection and for a score ≥5 sepsis or infection is very likely (RODWELL & al [12]).

Serial estimation of C-reactive protein (CRP) was measured by latex agglutination test in suspected cases of septicaemia or other infections. We considered an abnormal value > 6 mg/dl for interpretation.

Maternal risk factors considered were: preterm rupture or prolonged rupture of membranes, maternal *group B streptococcus* colonization (especially if untreated during labor), maternal urinary tract infection, chorioamnionitis.

Aerobic and anaerobic blood culture using Signal System was done; after 24 hours incubation gram stains were done followed by blind subculture on 5% sheep blood agar, chocolate agar, and Levin agar. Both cultures were further preincubated and then subcultured after 48 hours then after 96 hours with last subculture on day 7. Identification of bacteria was made by conventional and biochemical methods: gram stain, catalase reaction, coagulase reaction, hemolytic activity on sheep blood agar plates and morphology on Levin agar, triple sugar iron agar reaction, indole, motility, citrate, urease, hydrogen sulphide production. API 20E identification kits (bioMerieux) were also used to confirm the identification of Gram-negative isolates. Antimicrobial susceptibility testing of all bacterial isolates was performed by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar according to the recommendations of the Clinical & Laboratory Standards Institute (CLSI).

We performed descriptive analysis and analytic analysis with chi-square test for comparison of groups, p value ≤ 0.05 being considered statistically significant.

Table 1. Patients characteristics

	Total	**Proven sepsis (n=98)**			**Unproven sepsis (n=387)**		
		Early onset (n=62)	Late onset (n= 36)	p-value	Early onset (n=264)	Late onset (n=123)	p-value
Gender							
Male	288	43	11		169	65	
Female	197	19	25	0.0001	95	58	0.03
Gestation							
Preterm	341	47	13		192	89	
Term	144	15	23	0.0001	72	34	0.93
Birth weight							
SGA	284	43	15		162	64	
AGA	201	19	21	0.007	102	59	0.08
Mode of delivery							
LSCS	137	18	12		73	34	
SVD	348	44	24	0.65	191	89	0.99
Maternal risk factors							
yes	364	54	25		182	103	
no	121	8	11	0.03	82	20	0.002
Overall mortality		16.12%	2.17%		6.81%	4.88%	0.05
		11.22%			6.20%		

*AGA - appropriate for gestational age; SGA - small for gestational age

*LSCS - Caesarean section; SVD - Spontaneous Vaginal Delivery

Results and Discussion

Among 485 neonates with clinical signs of sepsis, blood culture positivity was found in 7.83% (38 cases). Other than blood positive cultures (urine, body surface cultures, cerebrospinal fluid, and gastric aspirate) with clinical sepsis screening and haematological positivity were found in 60 of 485 cases (12.37%). We considered these two groups (98 cases) clinically and laboratory proven sepsis. Bacteriological negativity, sepsis screening negativity but clinical course compatibility with sepsis was found in 387 neonates, they were classified as clinical sepsis or unproven sepsis.

Out of 485 cases, 67.21% had early onset sepsis and 32.78% had late onset one. Male to female ratio was 1.46. In both groups predominates the early onset of sepsis. Preterm neonates represent 70.30% of our studied group. Small for gestational age occurs in almost one half of the cases (58.55%). Delivery was mostly spontaneous vaginal

in 71.75% of cases. Maternal risk factors were documented in 74.84% of cases. Survival rates were higher in group of unproven sepsis. Mortality was increased in early onset sepsis being encountered in preterm and SGA (Table 1).

The most prevalent clinical features observed were acute respiratory distress syndrome and temperature dysregulation (hypothermia-hyperthermia), followed by difficulties of feeding and jaundice (Table 2).

Total white blood cell count had little value and poor positive predictive accuracy as well as low platelet count, in accordance to other studies. Abnormalities in the CBC were found in 370 cases (76.28%), 27.21% neonates having leukocytosis, 49.07% leukopenia and 49.89% thrombocytopenia. Hematologic score (sepsis screen parameters) more than 4 was determined in 63.91%. In the present study CRP was abnormal in 48.86% of cases. Positive CRP test (≥6 mg/l) was found in 43 of 98 proven cases and in 194 of 387 unproven cases (Table 3).

Table 2. Clinical signs of the neonates

	Total	**Proven sepsis (n=98)**			**Unproven sepsis (n=387)**		
		Early onset (n=62)	Late onset (n= 36)	p-value	Early onset (n=264)	Late onset (n=123)	p-value
Jaundice							
yes	330	53	25		182	70	
no	155	9	11	0.05	82	53	0.02
Respiratory distress							
yes	350	52	22		197	79	
no	135	10	14	0.01	67	44	0.03
Difficulties to feeding							
yes	339	56	27		184	72	
no	146	6	9	0.04	80	51	0.03
Lethargy							
yes	172	23	13		91	45	
no	313	39	23	0.92	173	78	0.68
Hypothermia-hyperthermia							
yes	338	35	20		181	102	
no	137	27	6	0.006	83	21	0.002
Convulsions							
yes	155	28	17		78	32	
no	330	34	19	0.84	186	91	0.47
Vomiting							
yes	138	26	16		69	27	
no	347	36	20	0.80	195	96	0.37
Excessive crying							
yes	348	41	22		189	96	
no	137	21	14	0.61	75	27	0.17

In our group, more than one half of the identified microorganisms (53 from 98) were gram-positive and 45 were gram-negative. *Candida species* were not isolated. The most common microorganisms isolated were *Coagulase-negative staphylococci*, especially in early onset sepsis, followed by *E. coli* isolated especially in late onset. The first ones were the most common gram-positive bacteria isolated, 18 of them and 5 *Staphylococci aureus* were found to be methicillin-resistant. Distribution of microorganism is shown in Table 4.

E. coli was found to be sensitive to amoxicillin-clavulanic acid, piperacillin-tazobactam, cefuroxime, ceftazidime, gentamicin, quinolones and meropenem with the highest degree. None of the gram-negative isolates were beta-lactamase or carbapenems-producing. *Staphylococci* species isolated were found to be sensitive to amoxicillin-clavulanic acid, cefuroxime, vancomycin, clindamycin, teicoplanin and quinolones with a rate of 100% and resistant to amoxicillin and penicillin. Antibiotic sensitivities of microorganisms are shown in Table 5.

Improvement of technical features of neonatal intensive care units, innovations in treatment and practices increases the chance of survival for preterm and small for gestational age babies. Despite this, long hospitalization periods and immaturity of the immune system of these babies cause different infections, including sepsis.

During the six years study period, 485 neonates with suspected neonatal sepsis (using clinical criteria) were enrolled. The facts that the immune system of preterm and small for gestational age babies is immature causes infections to have a more severe course and sometimes leads to death. That is why all the positive cultures (urine, cerebrospinal fluid, gastric aspirates) have been considered when distributing patients to the proven sepsis group. Among these patients with proven sepsis, the disease was recognized as EOS in 62 of 98 cases and as LOS in 36 of 98 cases according to the onset of symptoms.

Among the entire group of 485 neonates, significant differences were seen about patient's sex, the overall male to female ratio being 1.46. Preterm and small for gestational age babies were more affected by septicaemia, in both groups EOS and LOS.

Clinical characteristics are useful for identifying children with septicaemia, but they have limited specificity

and sensitivity (WEBER & al [13]). In our study, significant clinical findings included hypothermia-hyperthermia (especially hypothermia), respiratory disorders, and feeding difficulties. The association between maternal infections (urinary, vaginal) during labour and development of EOS is well known, demonstrating significant statistical association.

Table 3. Results of evaluation by scoring hematologic system and CRP

	Total	Proven sepsis (n=98)		Unproven sepsis (n=387)		
		Early onset (n=62)	Late onset (n=36)	Early onset (n=264)	Late onset (n=123)	p-value
Leukopenia	132	27	14	60	31	0.0005
Leucocytosis	238	34	17	163	24	0.99
Thrombocytopenia	242	23	15	121	83	0.41
Score system ≥ 4	310	46	23	193	48	0.006
Positive CRP	237	26	17	152	42	0.014

Table 4. Bacteriological profile in culture–positive cases

Organisms	Late onset sepsis number (%)	Early onset sepsis number (%)	Total
Gram positive bacteria	19(52.77%)	34(54.83%)	53(54.08%)
S. aureus	6(16.66%)	6(9.67%)	12(12.24%)
Coagulase negative staphylococci	13(36.11%)	27(43.54%)	40(40.81%)
Enterococcus faecalis	-	1(1.61%)	1(1%)
Gram negative bacteria	17(47.22%)	28(45.16%)	45(45.91%)
E. coli	15(41.66%)	21(33.87%)	36(36.73%)
Enterobacter spp	2(5.5%)	7(11.29%)	9(9.18%)
Total	36	62	98

Complete blood cell count is difficult to interpret in the neonatal period because it varies significantly with day of life and gestational age. Total and differential leukocyte count, and platelet count, when combined have been found to be very useful to diagnose neonatal sepsis before result of blood culture (MANROE & al [11]), but recently poor sensitivities and specificities have been associated with them (BHAT & al [14]).

A considerable proportion of neonatal bloodstream infections had a normal or low initial CRP level, which was more likely to occur in low birth weight or extremely preterm infants, those with earlier onset sepsis, and those infected with *CoNS* (LAI & al [15]). Rapid techniques like C-Reactive Protein (CRP) assays may help in the diagnosis of septicaemia, but they lack the capacity to detect specific pathogens and the diagnostic accuracy of CRP in diagnosis of neonatal sepsis was 70.07% (EFFAT & al [16]).

Haematological score of 3-4 and a level of CRP>10 mg/L in blood culture negative cases would be an effective guide to make decisions regarding judicious use of antibiotic therapy, representing quickly and readily available tools in diagnosing neonatal sepsis (USHA & al [17]). In our study, significant laboratory findings associated with sepsis included leukopenia, a score system more than 4 and increased CRP.

Blood culture is still the gold standard for definitive diagnosis of neonatal sepsis, but is time consuming, it has low sensitivity, and contamination is possible especially with commensally Coagulase negative staphylococci. Neonatal blood culture positive rate has been found to range from 25-60% (JYOTHI & al [18]). Low rates were reported in the developed countries that can be explained by the good quality of life, health care, education and hospital services. Intrapartum antibiotic prophylaxis has decreased the incidence of early onset infections. In our study, the rate of bloodstream infection was found to be 7.83% and it was noted that the rate was lower than the rates reported in the literature. Multiple blood cultures could help increase the diagnostic accuracy of the test. The most common cause of early-onset sepsis is *Group B streptococcus* (GBS), followed by *Escherichia coli*. The remaining cases of early-onset sepsis are caused by *Staphylococcus aureus, coagulase negative staphylococci*, *Listeria monocytogenes* and other gram-negative bacteria. In very low birth weight new-borns *E. coli* is more common than GBS (VERGNANO & al [19]; STOLL & al [20]). The main pathogen in late-onset sepsis is CoNS, followed by *S. aureus, E. coli*, *Klebsiella pneumoniae* and *Candida species*. Less common causes of late-onset sepsis include *Enterococcus* species and *Pseudomonas aeruginosa* and they are more resistant to antibiotics than early-onset pathogens (BIRJU & al [21]).

In our study, the incidence of neonatal sepsis in both EOS and LOS was predominantly associated with gram-positive cocci, specifically CoNS, similar findings were obtained in other studies. In some studies, CoNS were more common causes of LOS (BIRJU & al [21]), contrary to our results. Despite the importance and role of CoNS as etiological agents of neonatal sepsis as proved in many studies, determination of the identity of CoNS isolates whether being true pathogens or contaminants is still problematic. The same problem is also encountered in case of urinary infections caused by CoNS other than *S. saprophyticus* and *S. aureus (*CRISTEA & al [22]). In our study, *S. epidermidis* was the most frequently CoNS isolate in

blood cultures, followed by *S. haemolyticus*. A number of 18 from 40 were resistant to methicillin. EFIRD & al [23] reported that CoNS was the most commonly isolated microorganism and *Klebsiella pneumonia* was the second most commonly isolated microorganism in eight NICU's in USA. A blood culture positive for *Staphylococcus aureus* is always a true bacteraemia and we have demonstrated it to be a cause of LOS in 16.66% of cases.

Table 5. Antibiotics sensitivity patterns of isolates

Antibiotics	***E. coli***	***Enterobacter cloacae***	***S. aureus***	***CoNS***	***Enterococcus faecalis***
Amoxicillin	0%	10%	0%	0%	100%
Amoxicillin-clavulanic acid	100%	100%	100%	100%	100%
Piperacillin-tazobactam	100%	100%	-	-	-
Oxacillin	-	-	75%	58.34%	-
Cefoxitin	-	-	58.33%	55%	-
Cefuroxime	100%	100%	100%	100%	100%
Penicillin	-	-	16.3%	21.3%	100%
Ceftriaxone	74.28%	100%	-	-	-
Ceftazidime	100%	100%	-	-	-
Meropenem	100%	100%	-	-	-
Vancomycin	-	-	100%	100%	100%
Teicoplanin	-	-	100%	100%	100%
Clindamycin	-	-	100%	100%	100%
Gentamicin	100%	100%	-	-	-
Amikacin	100%	100%	-	-	-
Erythromycin	-	-	75%	86.48%	-
Clarithromycin	-	-	75%	81.08%	-
Ciprofloxacin	100%	100%	100%	100%	100%
Ofloxacin	100%	100%	100%	100%	100%

Gram-negative bacteria were the second cause of neonatal sepsis especially *E. coli*. Other Gram-negative bacilli (*Enterobacter spp*) were recovered but in a few numbers. These data are consistent with scientific literature data (CORTESE & al [24]).

The difference between negative and positive gram-negative etiology is minimal; if we would exclude cases with CoNS as etiological agent, it resulted that the main etiological agent was *E. coli* (36 isolated from 98).

Antimicrobial resistance increases the morbidity and mortality in hospital contributing to rising costs of care, prolonged hospitalization and need for more expensive drugs in children and adults (JURCĂ & al [25]; ZAHA & al [26])

Ampicillin and aminoglycosides (mainly gentamicin) are the first-line empirical antibiotics used in NICUs. Quinolones (ciprofloxacin) are not recommended for use in young children. In our study, all staphylococcus aureus and CoNS isolates showed high resistance to amoxicillin and penicillin, and sensitivity to amoxicillin-clavulanic acid, cefuroxime, and quinolones. The sensitivity of different CoNS species and *Staphylococcus aureus* to oxacillin, erythromycin and clarithromycin was variable. All staphylococcal isolates were sensitive to glicopeptides as previously found in other reports, but their over prescription may result in the development of vancomycin-resistant strains such as enterococci.

E. coli was highly sensitive to amikacin, gentamycin, amoxicillin-clavulanic acid, cefuroxime, ceftazidime, meropenem, followed by quinolones and to a low degree to ceftriaxone. *Enterobacter cloacae* was sensitive to all antibiotics tested with except of amoxicillin. AURANGZEB & al. [25] reported that gram negative microorganisms in NICU's were resistant to ceftazidime to a rate of 19.4% and resistant to cephotaxim to a rate of 44.8%. In our study, *E. coli* and *Enterobacter cloacae* had a lower resistance against the third generation cephalosporins.

The overall mortality rate for proven neonatal sepsis was estimated as 11.22% almost twice than for unproven one. Moreover, mortality was more common in preterm, small for gestational age new-borns in the early onset sepsis group.

Conclusion

The clinical diagnosis of neonatal sepsis is difficult because the signs and symptoms are not specific, early diagnosis and prompt treatment remaining a challenge. There is no laboratory test with 100% sensitivity and specificity. Bacteria are only identified on Gram stain and speciation and susceptibility results take an additional 24-48 hours after a culture is reported as positive. Moreover, in many cases blood cultures fail to detect the causative organism or there is a subset of patients with positive blood cultures due to contamination. Contamination of blood culture specimens submitted to the microbiology department is not uncommon and differentiating it from true bacteraemia is sometimes difficult. Identification of bacteraemia is only a part of the patient evaluation because it is essential to identify the source of bacteraemia.

The combination of parameters (sepsis screening) yielded better results than single tests and proved to be a valuable aid for early diagnosis of neonatal sepsis. Mortality in early onset sepsis was relatively higher. Early onset sepsis was more frequent than late onset sepsis. The common predisposing neonatal factors for early onset sepsis are prematurity, low birth weight and maternal risk factors.

The predominant isolate was *Coagulase negative staphylococcus* consistent with other reports. The clinical significance of this result, considering the low virulence of CoNS is difficult to interpret. The resistance of gram-positive bacteria against vancomycin and methicillin and the resistance of gram-negative bacteria against third generation cephalosporin have led to significant concern in terms of antibiotic selection. Our study has shown lower antibiotic resistance compared to the rates reported in the literature caused by limited use of glycopeptides and third generation cephalosporin in our unit.

Despite efforts to diagnose and manage sepsis, this condition remains an important public health issue. Establishing a structured diagnosis algorithm to allow rapid recognition of sepsis and prompt initiation of treatment are important goals in reducing specific morbidity and mortality and, implicitly, infant mortality.

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