ORIGINAL ARTICLE

Bacterial invasive infections in a neonatal intensive care unit: a 13 years microbiological report from an Italian tertiary care centre

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Keywords

NICU • Invasive infections • Colonization • *Staphylococcus aureus*

Summary

Introduction. *To evaluate the aetiology of neonatal invasive diseases (positive cultures from blood or cerebrospinal fluid, CSF) due to bacteria other than coagulase-negative staphylococci in a large tertiary care centre and compare with results of surveillance cultures.*
Methods. *Retrospective analysis of microbiological data of children admitted in neonatal intensive care unit (NICU) of a large tertiary care centre from 2005 to 2018.*
Results. *230 bacterial strains, 223 from blood and 7 from CSF, respectively, were detected as cause of invasive infections, while 152 were detected in surveillance cultures. Methicillin-susceptible Staphylococcus aureus (MSSA) was the most frequently isolated pathogen both in invasive infections (18%) and colonizations (23%) followed by Escherichia coli (16% on invasive disease and 20% of colonizations). Other common bacteria include Enterococcus faecalis and Streptococcus agalactiae for invasive disease and methicillin-resistant Staphylococcus aureus in colonizations. Invasive infection was due to a pathogen detected in surveillance cultures in 33% of cases. In more than 50% of invasive diseases the identified pathogen was not present in surveillance cultures.*
Conclusions. *The high percentage of invasive infections due to bacteria not previously identified in surveillance cultures raises doubts about the efficiency of this procedure and highlights the need to search for alternative infection sources. This finding and the high prevalence of invasive infections due to nosocomial pathogens such as Staphylococcus aureus could be the result of horizontal transmission between patients through the hands of health care professionals, emphasizing once again the importance of applying stringent hand hygiene procedures and isolation standards.*

Introduction

Invasive infections are a leading cause of morbidity and mortality in pre-term infants with wide differences between countries [1]. Despite the lack of a consensual definition for neonatal sepsis, this clinical condition is usually considered in the presence of a positive culture from blood or cerebrospinal fluid [2], associated with different clinical signs or symptoms, that might be non-specific. Initial symptoms might be few and could include apnoea or tachypnoea or temperature variations. Later on, signs of poor perfusion (as pallor and/or mottled skin) associated with tachycardia or bradycardia may appear. Respiratory symptoms include apnoea, distress or cyanosis. On the neurological side irritability or lethargy could be frequently seen [3].
Neonatal sepsis is traditionally divided in early onset (occurring in the first 72 hours of life) and late onset (occurring after 72 hours) with different pathogens involved. The most common agents associated with early-onset neonatal sepsis are *Streptococcus agalactiae* (group B streptococci, GBS) and *Escherichia coli*. Both are pathogens that typically colonize the maternal genitourinary tract and may infect the newborn whether in utero or during delivery. *Listeria monocytogenes* and non-typeable *Haemophilus influenzae* have also been implicated in early-onset neonatal sepsis, although less frequently [4]. Late onset sepsis recognise a higher prevalence of Gram-positive bacteria (*Staphylococcus aureus* and coagulase-negative staphylococci, CoNS) as a result of postnatal exposure to healthcare: contacts with hospital staff, contaminated devices (mainly central catheters in preterm infants) and parents [3, 5].
The aim of this study was to report microbiology of invasive infections and colonizations by bacteria other than common skin contaminants in patients admitted in a neonatal intensive care unit (NICU).

Materials and methods

Istituto Giannina Gaslini, Genoa, Italy, is a children's care hospital in northern Italy serving as local paediatric hospital for the Genoa area and also representing a tertiary care centre for the whole Italy and many foreign countries. According to the Italian law, to be defined as "third level", paediatric and neonatological functional units must have no less than 1000 births/year, a catchment area of at least 5000 births/year and accept births that require any kind of assistance including intensive care.

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Microbiological data from patients admitted in NICU, from January 2005 to October 2018 were anonymously extracted from the Laboratory of Microbiology database, according to Istituto Gaslini data protection policy based on European Union Data Protection Rules (https://ec.europa.eu/commission/priorities/justice-and-fundamental-rights/data-protection/2018-reform-eu-data-protection-rules_en). As a consequence, demographic and clinical data could not be retrieved, but only data on ward of admission and site of isolation were available.

In the NICU the surveillance cultures protocol calls for nasal, pharyngeal and rectal swab, and tracheal aspirates in those who are invasively ventilated [6] to detect potentially pathogens, including carbapenem-resistant enterobacteria [7]. These cultures are performed at time of 1st admission and then repeated weekly. For the purpose of the present study in case of multiple isolations of the same pathogen in a single patient only the first one isolation was considered. In case of clinical suspicion of invasive disease blood cultures and cultures from clinically relevant sites, included cerebrospinal fluid (CSF) are performed. Invasive infections were defined by isolation of pathogens from blood or cerebrospinal fluid diagnosed in presence of a positive blood or CSF culture.

Blood or CSF cultures yielding CoNS were excluded from the analysis since the lack of clinical data on patients implied the impossibility to define a real infection (invasive or localized) rather than a bacterial contamination.

Statistical analysis

Categorical variables were reported as proportions (percentages, %), while continuous variables were described in terms of median and inter quartile range (1st and 3rd quartile, IQR) because of their non-normal distribution. The epidemiology of bloodstream infections and colonizations was analysed by calculating the rate of episodes, i.e. the number of invasive infections or first episode of colonization observed in one year divided by the total number of admissions in the same period and expressed as episodes/1,000 admissions. The evaluation of changes in rates during the study period were performed with the Pearson's correlation coefficient (r) that is a measure of linear association between two variables. To test whether the association between year and isolation rates was merely apparent and might have arisen by chance we used the t test in the following equation: $t = r\sqrt{[(\text{number of observations} - 2 \text{ degrees of freedom})/(1-r2)]}$. The t value was compared with specific tables of two-tailed distribution, and a $P \leq 0.05$ was considered as significant [8]. Calculations of r and t coefficients were performed by means of Microsoft Excel 365 for Windows (Microsoft Corporation 2019).

Results

During the study period 230 bacterial strains fulfilled our inclusion criteria: 223 from blood and 7 from CSF, detected in 198 patients. A total of 152 strains were detected in surveillance cultures in 61 patients.

Table I reports on absolute numbers and proportions of different pathogens causing invasive infections or colonizations. Methicillin-susceptible *Staphylococcus aureus* (MSSA) was the commonest isolated pathogen in invasive infections (17.8%) followed by *Escherichia coli* (16.5%), *Enterococcus faecalis* (14.8%) and *Streptococcus agalactiae* (10%). Among Gram-negatives causing disease carbapenem resistance was observed in 1 *Pseudomonas aeruginosa* strain, but in no case of invasive infection due to *Enterobacteriales*.

MSSA was the most frequently identified pathogen (23.2%) also in case of colonization, followed by *Escherichia coli* (19,9%) and methicillin-resistant *Staphylococcus aureus* (MRSA) (11.3%). None of Gram-negatives colonizing patients resulted carbapenem resistant.

A total of 76 strains (33% of those observed in invasive diseases) were detected both in blood and surveillance cultures: for 56 bloodstream infection followed colonization detection after a median of 7 days (IQR 2.5-21.5), while for other 20 (8.6%) blood and surveillance cultures resulted positive on the same day. Other 42 strains (18.3%) causing invasive infections were not present among bacteria already colonizing the patient. Finally, 116 (50.0%) strains were isolated in absence of any colonization.

Table II reports the yearly rate of invasive infections during the study period and the rate of infections due to pathogens colonizing the patient.

Mean crude rate of invasive infection was 73 with a minimum of 27 and maximum of 168.7 in 2012. Mean rate of sepsis by the same pathogen from a previous colonization was 23.5 with a maximum of 40 in 2016. During the study period there was an increase in the rate of invasive infections. The analysis of possible correlations between rate of bloodstream infections or colonizations and year of observation showed t values of 0.377 and 1.72, respectively, without significant differences.

Discussion

In the present study we analysed the epidemiology of invasive bacterial infections due to pathogens other than "common" skin contaminants, in neonates admitted in NICU in a tertiary care Italian centre. A colonizing agent was the cause of 33% of invasive infections, with a median of 7 days between colonization and disease occurrence, and in less than 10% of cases the same agent was detected in blood and surveillance cultures collected on the same day. Noteworthy near 20% of diseases was due to non-colonizing microorganisms and in ½ of cases of bacteraemia it was observed in absence of any colonization. As a consequence, we could estimate that surveillance cultures detect only 1/3 of pathogens that will cause invasive disease in our NICU.

Tab. I. Bacterial prevalence in invasive infections and colonizations (n = absolute number).

Pathogen	Pathogens isolated in invasive infections (total 230)		Pathogens isolated in surveillance cultures (total 152)		Concomitant (same day) isolations in surveillance cultures and invasive disease (total 72)	
	N	% over total positive blood or CSF cultures	N	% over total colonizations	N	% over total invasive diseases
Methicillin susceptible *Staphylococcus Aureus*	41	17.8	35	23.0	19	8.3
Escherichia coli	38	16.5	30	19.7	17	7.4
Enterococcus faecalis	34	14.8	4	2.6	1	0.4
Streptococcus agalactiae	23	10.0	4	2.6	4	1.7
Klebsiella pneumoniae	20	8.7	11	7.2	6	2.6
Methicillin resistant *Staphylococcus Aureus*	19	8.3	17	11.2	9	3.9
Klebsiella oxytoca	15	6.5	7	4.6	2	0.9
Enterobacter cloacae	11	4.8	10	6.6	3	1.3
Serratia marcescens	5	2.2	7	4.6	3	1.3
Enterobacter aerogenes	4	1.7	3	2.0	1	0.4
Enterococcus spp.	3	1.3				
Enterococcus faecium	2	0.9				
Haemophilus influenzae	2	0.9				
Listeria monocytogenes	2	0.9	1	0.7	1	0.4
Morganella morganii	2	0.9				
Citrobacter koseri	1	0.4	2	1.3	2	0.9
Citrobacter spp.	1	0.4				
Enterobacter spp.	1	0.4	1	0.7	1	0.4
Klebsiella spp	1	0.4	1	0.7	1	0.4
Proteus mirabilis	1	0.4	1	0.7	1	0.4
Pseudomonas aeruginosa	1	0.4	4	2.6		
Serratia plymuthica	1	0.4				
Stenotrophomonas maltophilia	1	0.4	4	2.6		
Streptococcus pneumoniae	1	0.4	1	0.7	1	0.4
Acinetobacter baumannii			4	2.6		
Bacillus cereus			2	1.3		
Citrobacter freundi			1	0.7		
Serratia liquefaciens			1	0.7		
Serratia spp.			1	0.7		

Tab. II. Year-on-year trend in invasive infection in NICU (n = absolute number).

	Admissions (N)	Invasive infections (N)	Rate	Invasive infections by the same pathogen previously colonizing the patient (N)	Rate
2005	67	4	59.7	1	14.9
2006	277	17	61.4	9	32.5
2007	197	11	55.8	7	35.5
2008	90	11	122.2	2	22.2
2009	234	14	59.8	5	21.4
2010	258	7	27.1	1	3.9
2011	230	14	60.9	3	13.0
2012	166	28	168.7	5	30.1
2013	191	13	68.1	4	20.9
2014	253	11	43.5	6	23.7
2015	280	15	53.6	5	17.9
2016	273	21	76.9	11	40.3
2017	312	13	41.7	6	19.2
2018	213	26	122.1	7	32.9

N: absolute number; rate: episodes/1,000 admissions.

Invasive disease can follow colonization because of barrier leak, an event related with pathophysiology of neonate (e.g. immaturity of skin and gut barriers) influenced by iatrogenic factors such as insertion and/or manipulation of devices (e.g. central venous catheters) or therapies (e.g. use of proton pump inhibitors) [9]. On the other hand, bacteremia from non-colonizing pathogens could derive from cross transmission between patients in the same ward, and the transfer may not only take place directly via healthcare workers hands, but also indirectly through contamination of the patient-unit. In departments with high-intensity care and long hospital stays such as NICUs, this problem is particularly important [10]. It is likely, but not directly provable, that this type of transmission was one of the main causes of the majority of invasive infections observed in our study. MSSA was the most commonly colonizing pathogen associated with invasive infection, and this observation is similar to that reported in other third-level centres [11, 12]. The 26% prevalence of *S. aureus* (MSSA and MRSA) infections we observed in our study is higher than that observed in other series [13, 14], but there may be differences related to local factors that could affect the results. Interestingly, we observed also a non-negligible proportion of GBS (10% of all invasive infections and 2.6% of surveillance cultures) as a cause of late infection, confirming our previous results [15]. In this case we can hypothesize a role of maternal late colonization transmitted via breast milk [15].

As regards Gram-negatives, the most frequently isolated pathogen in invasive diseases was *Escherichia coli* (16%) followed by *Klebsiella pneumoniae* (9%) and *Klebsiella oxytoca* (6%). These proportions are different to that reported in other studies [16-18], but also in this case local factors could represent the leading cause for the observed discrepancies.

Beyond the relationship between colonization and infection in a given patient, there is the problem of horizontal transmission (i.e. cross infections by healthcare workers hands). A single study sought to create a model of indirect transmission between patients through the hands of healthcare workers in a context completely different and not comparable with a NICU (adult surgery unit) [19]. Hand hygiene is the first rule to be applied in order to interrupt the chain of contamination (both of patient-unit and directly to the patients) and infection: the aforementioned study [19], clearly documented that the proportion of hand hygiene procedures adherence is inversely proportional to pathogens transmission. In a study conducted in our Institution we estimated the average number of hand hygiene procedures in different wards including NICU that showed a very high number of correct procedures [20]. In spite of this, it is likely, but not directly provable (given the limits of our methods relying solely on culture data and not molecular typing), that the majority of invasive infections observed in our study could have been due to pathogens not colonizing the patient but brought in some way by the hands of the staff, by means of a not correct approach [17].

Finally, the efficiency and role of surveillance cultures for identification of pathogens causing invasive diseases in NICU is still to be completely determined. The problem is not easy to solve since it has been proved that interventions aimed at promoting hand hygiene alone may not be sufficient to reduce the incidence of some pathogens, in particular multi-resistant ones [17]. Therefore, isolation procedures and correct hand hygiene must be implemented and constantly maintained especially in high risk wards as NICU [20].

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

MM collected data, analyzed results and drafted the manuscript. RB designed the study, collected data and reviewed the manuscript. DLM analyzed results and reviewed the manuscript. DM collected data a, analyzed results and reviewed manuscript. IB, SS, AM and CS analyzed results and reviewed the manuscript. LR designed the study and reviewed the manuscript. EC designed the study, analyzed results and reviewed the manuscript. All authors critically reviewed the manuscript and approved the final version.

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