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Original Research Article

Neonatal sepsis in a tertiary care hospital in Delhi, India: study of microbial profile and antimicrobial susceptibility pattern

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ABSTRACT

Background: Sepsis is one of the most common cause of neonatal deaths globally more so in low and middle-income countries. The key to management is high degree of clinical suspicion and prompt initiation of empirical antibiotic therapy pending investigations' results. Knowledge of one's own NICU flora and antimicrobial susceptibility pattern guides in choosing correct antibiotic therapy to pediatrician. If this data is standard and comparable across different sites, then it also helps in formulating regional and National treatment guidelines. Present study was therefore undertaken to study microbial flora of present NICU and analyze their antimicrobial susceptibility pattern and formulate antimicrobial policy.
Methods: Data of blood culture isolates sent from suspected cases of neonatal sepsis received from January 2017 to July 2018 was analysed by "WHONET".
Results: One hundred ninety-three non-repeat isolates were obtained from 992 blood culture samples. Coagulase negative Staphylococcus and *K. pneumoniae* were the most common isolates. Non albicans Candida were responsible for majority of fungal infection. There was an outbreak of *C. pelliculosa* for six months. Most of the bacteria were multidrug resistant (MDR). However, except one all other Candida isolates were sensitive to antifungal drugs.
Conclusions: WHO guidelines suggest use of penicillin and gentamicin for neonatal sepsis. But in present study, they were not found useful, instead amikacin, netilmicin and piperacillin-tazobactam were found useful and changes were made in antibiotic policy. Authors therefore recommend regular monitoring of antimicrobial susceptibility pattern followed by necessary changes in antibiotic policy for reasonable empirical therapy.

Keywords: Antibiotic susceptibility, CONS, Candida, *Klebsiella pneumoniae*, Neonatal sepsis

INTRODUCTION

Neonatal sepsis is a significant cause of morbidity and mortality among neonates worldwide.[1,2] WHO has estimated that 1.6 million deaths occur globally every year due to neonatal infections and 40% of all neonatal deaths occur in developing countries.[3] In India, the incidence of blood culture proven sepsis was reported as 8.5 per 1,000 live births for the year 2002-2003 by the National Neonatal Perinatal Database.[4] The key to management is clinical suspicion, early diagnosis and starting correct empirical antibiotic therapy at the earliest.[5] This is well proven that each NICU has its own unique microbial flora. Hence in a NICU setting, empirical treatment of sepsis before the culture reports become available depends on the knowledge of local microbial flora of NICU and its antimicrobial susceptibility pattern. Further if culture and antimicrobial susceptibility testing is carried out by using all quality checks and data is analyzed using a globally approved

software like "WHONET" the results can be compared nationally and internationally. Such data is also extremely important for formulation of both hospital level and national level guidelines and antibiotic policies. The current study was therefore undertaken with the primary objective to know local microbial flora of present NICU and its antimicrobial susceptibility pattern. The secondary objective was to generate a globally standard, shareable and comparable database which will help policymakers at both local and national level to formulate correct antibiotic treatment in cases of neonatal sepsis and interventions to reduce nosocomial infections in NICU.

METHODS

This was a retrospective study conducted jointly by Microbiology and Pediatrics department of ESIC PGIMSR Basaidarapur, New Delhi for a period of 18 months from January 2017 to June 2018. For the above study period authors analyzed results of all blood cultures sent from NICU of present hospital. These samples were collected from neonates who were suspected of neonatal sepsis in BacT/Alert® PF Plus blood culture bottles and sent to Microbiology laboratory for processing. Samples were processed in bioMérieux BacT/Alert 3D® automated blood culture system. This system is capable of detecting all bacteria (aerobic and facultative anaerobic) and yeasts from blood culture samples. Samples were incubated for up to 5 days before labeling them as sterile. All positive samples were processed by standard bacteriological methods to obtain growth of bacteria and yeasts. Further identification was done manually and/or by Vitek 2® compact automated system. Antimicrobial susceptibility of bacterial isolates was tested by disk diffusion method as per Clinical and Laboratory Standards Institute guidelines and/or Vitek 2® compact system using AST cards.[6-8] E test® was done to confirm minimum inhibitory concentration (MIC) of vancomycin and colistin using E test strips from Biomerieux, France. In disk diffusion tests cefoxitin disks were used to detect MRSA.

Quality assurance

Stringent quality control procedures were used at each step as per CLSI documents for manual tests and kit/manufactures' instructions for Vitek ID/AST system and E Test using ATCC strains of various bacteria and *Candida spp*. Apart from this, present laboratory continuously participated in EQAS Microbiology conducted under the aegis of IAMM by SGRH Delhi and EQAS Mycology by PGI Chandigarh**.**

Statistical analysis

All the data was entered, calculated and analyzed by using "WHONET" software (version 2018) made freely available for online download by world health organization (WHO). Bacterial susceptibility data was interpreted using breakpoints given in current CLSI guidelines and reported as susceptible, intermediate or resistant for each individual antibiotic.[8] For antifungal susceptibility species specific breakpoints given by CLSI are used.[9] Since for *C. pelliculosa* no such breakpoints were available hence it was interpreted by C. albicans breakpoints. From a few patient's same isolate was obtained repeatedly in culture. For such cases, during calculation only first isolate with antibiotic susceptibility was taken into consideration so as to avoid reporting false high drug resistance. Wherever antimicrobial susceptibility of an isolate-drug combination was tested by more than one methods the preference for interpretation of results in "WHONET" was set as E Test>MIC>disk diffusion.

RESULTS

From January 2017 to June 2018, a total of 183 non-repeat bacterial and Candida isolates were obtained from 992 samples received from NICU. Coagulase negative Staphylococci (CONS) were the most common isolate closely followed by *Klebsiella spp*, each being responsible for about one third of total infections. Candida species were third most common isolate comprising of 16.4% of total. Thus, these three isolates together were responsible for about 80% of all infections (Table 1).

Table 1: Microbial profile of neonatal sepsis.

Microorganism	Number of isolates	Percentage of isolates
Gram negative bacteria		
Escherichia coli	3	1.6
Klebsiella spp.	57	31.1
Enterobacter cloacae	2	1.1
Citrobacter spp.	10	5.5
Serratia marcescens	1	0.5
Acinetobacter spp.	5	2.7
Pseudomonas aeruginosa	3	1.6
Gram positive bacteria		
Staphylococcus aureus	4	2.2
Staphylococcus, coagulase negative (CONS)	58	31.7
Enterococcus spp.	10	5.5
Fungal		
Candida spp.	30	16.4
Total	183	100.0

In present study, Gram negative bacteria (81) slightly outnumbered gram positive (72) isolates. Coagulase negative Staphylococci were the single most common isolate comprising of 31.7% of total with S. aureus causing only four cases (2.2%) of septicaemia. Most common CONS species was *S. epidermidis* followed by *S. haemolyticus*. Second most common isolate was Klebsiella species (commonest being *K. pneumoniae*). Enterobacter species and Citrobacter species together made another 6.5% of total isolates. Ten isolates of Enterococci were encountered four of which were *E.*

faecium and rest 6 were *E. faecalis*. Among non-fermenters, authors recovered five isolates of *Acinetobacter baumanii* and three *P. aeruginosa*. Fungal pathogens comprised of 30 isolates of Candida species. These 30 isolates were *C. pelliculosa* (19), *C. tropicalis* (8), *C. albicans* (2) and *C. glabrata* (1). Antimicrobial susceptibility profile of various isolates was as follows:

Staphylococci

Since the number of *S. aureus* isolates was insignificant (4/62), both *S. aureus* and CONS were grouped together for calculation of antimicrobial susceptibility results (after interpretation was done according to their species-specific breakpoints). Most staphylococcal isolates (>90%) were found resistant to penicillin and erythromycin (Table 2). Methicillin resistance rate in present study was very high with 82% of Staphylococci being methicillin resistant.

Susceptibilities to fluoroquinolones, clindamycin and trimethoprim/ sulfamethoxazole were also less than 50%. Aminoglycoside susceptibility was best for netilmicin (92.3%), followed by amikacin (76.9%) and least for gentamicin (42.9%). All methicillin resistant isolates were tested for vancomycin MIC by Vitek or E Test strips. All isolates were susceptible to vancomycin, teicoplanin and linezolid. None of vancomycin intermediate (VISA) or vancomycin resistant (VRSA) Staphylococcus isolate was encountered in present study.

Table 2: Antibiotic susceptibility profile of Staphylococcus species (n=62).

Antibiotic name	Number of isolates tested (n)	% Resistant	% Intermediate	% Susceptible
Penicillin G	62	90.3	0.0	9.7
Cefoxitin	62	82.0	0.0	18.0
Amikacin	59	17.9	5.1	76.9
Gentamicin	56	48.2	8.9	42.9
Netilmicin	56	0.0	7.7	92.3
Ciprofloxacin	51	72.5	5.9	21.6
Trimethoprim/ Sulfamethoxazole	61	49.2	3.3	47.5
Doxycycline	30	13.3	13.3	73.3
Erythromycin	58	93.1	0.0	6.9
Clindamycin	60	55.0	0.0	45.0
Linezolid	53	0.0	0.0	100.0
Vancomycin	59	0.0	0.0	100.0
Teicoplanin	59	0.0	0.0	100.0

Table 3: Antibiotic susceptibility profile of gram-negative enteric bacilli.

Antibiotic name	Number of isolates tested	% Susceptible	% Intermediate	% Resistant
Amoxycillin/Clavulanic acid	65	61.5	10.8	27.7
Cefoperazone/Sulbactam	61	93.4	1.6	4.9
Ampicillin/Sulbactam	56	83.9	8.9	7.1
Piperacillin/Tazobactam	72	94.4	1.4	4.2
Ceftazidime	68	27.9	2.9	69.1
Cefotaxime	63	30.2	0	69.8
Cefepime	68	35.3	17.6*	47.1
Cefixime	55	29.1	0	70.9
Aztreonam	69	31.9	7.2	60.9
Doripenem	31	96.8	0	3.2
Ertapenem	61	93.4	0	6.6
Imipenem	72	95.8	1.4	2.8
Meropenem	71	95.8	1.4	2.8
Amikacin	70	91.4	1.4	7.1
Gentamicin	63	33.3	0	66.7
Netilmicin	50	80	0	20
Ciprofloxacin	41	97.6	2.4	0
Trimethoprim/Sulfamethoxazole	72	29.2	0	70.8
Colistin	45	100	0	0

*SDD- Susceptible dose dependent

Enterococci

Total 10 Enterococci were isolated four of which were E. faecium and rest six were E. faecalis. Two of E. faecium were vancomycin resistant (VRE). These were confirmed by both E test and Vitek. Both had high level resistant to both vancomycin and teicoplanin. However, all isolates were linezolid susceptible. Seven out of 10 isolates were penicillin resistant and three of them also had high level gentamicin resistance.

Enterobacteriaceae

Total seventy-three non-repeat isolates belonging to family Enterobacteriaceae (enteric gram-negative bacilli) were isolated. Although WHO suggests using ampicillin and gentamicin for neonatal sepsis, it was not tested because most of Gram-negative isolates from present NICU and other hospital areas have been ampicillin resistant since 2015. Approximately Seventy percent of isolates in this study were resistant to third generation cephalosporins (cephotaxime, ceftazidime, cefixime) and aztreonam (Table 3). Cefepime was slightly better with resistance of 47% and another 17.6% isolates having dose dependent susceptibility. These bacteria also showed high resistance to trimethoprim-sulfamethoxazole (71%) and gentamicin (68%) despite the fact that former is not used in NICU. However, the other two aminoglycosides performed much better with netilmicin having 80% and amikacin having 91.4% susceptibility rates. Combination of a beta lactam inhibitor to beta lactams (BL/BLI) gave good results with susceptibilities rising to 60% for amoxycillin-clavulanic acid, 84% for ampicillin-sulbactam, 93.4% for cephoperazone-sulbactum and 94.4% for piperacillin- tazobactam. Although resistance was seen for carbapenems, still more than 90% isolates were susceptible to ertapenem, imipenem, meropenem and doripenem. None of the Enterobacteriaceae isolate was found to be colistin resistant except *S. marcescens* which is inherently resistant to colistin.

Nonfermenters

Out of five Acinetobacter isolates two were carbapenem resistant. These were also resistant to most of the other antibiotics. However, all nonfermenter isolates in present study were colistin susceptible. All three pseudomonas were carbapenem susceptible but two were MDR and had resistance to ceftazidime, piperacillin-tazobactam as well as aminoglycosides.

Candida

Candida isolates were tested for six antifungal drugs namely fluconazole and voriconazole among azoles, caspofungin and micafungin among echinocandins, amphotericin B and flucytosine. All candida isolates were susceptible to all the six antifungals drugs except one isolate each of *C. pelliculosa* and *C. glabrata*. Out of 19 single isolate of *C. pelliculosa* was resistant to amphotericin B and voriconazole. *C. glabrata* isolate was resistant to fluconazole and amphotericin B but was susceptible to both echinocandins.

DISCUSSION

Traditionally, the common pathogens implicated in neonatal sepsis include *E. coli*, group *B. streptococci*, *Listeria monocytogenes*, and *Enterococcus spp*. especially so in early onset sepsis.[5] However recent studies from low and middle income countries show different findings with a shift towards Gram negative bacteria like *E. coli*, Klebsiella, *S. aureus* and CONS in both early and late onset neonatal sepsis.[5-12] Also there are considerable local variations in microbial flora and drug susceptibilities. In present study authors found a high prevalence of multi drug resistant bacteria along with an outbreak of *C. pelliculosa* in NICU of present tertiary care hospital. In this study microbial flora of neonatal sepsis was made up of 44.2% Gram negative bacteria, 39.3% Gram positive bacteria and 16.4% Candida isolates. CONS made the single most common pathogen followed by *Klebsiella spp.* In one of the largest and recent multicenter study which was also conducted in three tertiary care hospitals of Delhi from 2011 to 2014, out of the total 1005 isolates of neonatal sepsis about two-thirds were Gram negative pathogens, the most common being *Acinetobacter spp.*, *Klebsiella spp.*, *Escherichia coli, Pseudomonas spp.*, and *Enterobacter spp.* The profile of isolates showed substantial variation among the three study sites. The most common isolate in the second site was *Klebsiella spp.* (89/359) and coagulase-negative staphylococcus (32/70, 46%) in the third site.[10] Klebsiella and CONS were also the most common pathogens in other Indian studies.[11-17] This type of microbial profile along with high antimicrobial resistance among these isolates clearly indicates most of these infections to be hospital acquired. Horizontal spread of infection due to poor hand hygiene among health care providers and patient attendants is possibly responsible for this. To a certain extent high CONS isolation rate could also be due to inappropriate sample collection as a result of unavailability of trained phlebotomists and open collection of blood culture samples using needle and syringe. Regular training in culture collection technique especially to new residents at the time of joining and use of closed blood culture collection system can probably reduce CONS isolation rate and give us a more realistic picture. It also emphasizes need of strengthening hand hygiene practices in labour room and NICU.

Like other studies authors also found high methicillin resistance rate among CONS but at 82%, this was alarmingly high and requires a rework-up of antibiotic guidelines and infection control practices in present NICU. Despite a high methicillin resistance, susceptibility was preserved for aminoglycosides like amikacin and netilmicin. Reason for this may be more frequent use of gentamicin which is usually started as the first line drug along with either a third-generation

cephalosporin or a BL/BLI combination for empiric treatment of neonatal sepsis. Authors didn't come across any linezolid resistant Staphylococcus in this study. Das et al reported similar high degree of sensitivity to linezolid.[13] However linezolid resistance from 13 to 29% has been reported by other authors.[12,16] Although no vancomycin resistant or intermediate Staphylococcus (VRSA/VISA) was reported but 2 out of 10 (20%) Enterococci were VRE. Denis study also reported VRE rate of 27% from another Delhi NICUs.[10] Like other Indian studies, authors observed a high cephalosporin resistance (47-70%) in all Gram negative isolates.[5,10,12,15,17] However carbapenem resistance was lower in present NICU with about 95% susceptibility in Gram negative enteric bacilli compared to others who have reported up to 55.6% *K. pneumoniae* resistant to meropenem.[13] This was also because of lesser prevalence of Acinetobacter in present NICU. Authors did not see any colistin resistant isolate which is a problem in many other tertiary care centers in India.[12,13,16] Das S et al, have reported decline in sensitivity to colistin over a two-year study period from 100% in phase 1 to 94.6% in phase 4.[13]

Candida spp. were found to cause thirty (16.4%) cases of neonatal sepsis in present study. All except one were non albicans Candida. Most common isolate was *C. pelliculosa*. However, *C. pelliculosa* is not a usual isolate from present NICU. This was an outbreak which lasted for about six months from July to December 2017 and could be controlled by active efforts of infection control team and also because all isolates were fluconazole sensitive. There have been a few case reports of outbreaks by this organism in NICU in literature.[18,19] During rest of the study period, C. tropicalis was the most common *Candida spp.* with 8 cases isolated over a period of 18 months. Samaga et al have reported Candida as a causative agent of 37.8% cases of neonatal sepsis.[15]

On the basis of above results, authors changed present empiric therapy from gentamicin to amikacin or netilmicin and/or piperacillin-tazobactam. This empirical therapy has worked well for last six months in present NICU for management of neonatal sepsis. The antibiotics are further deescalated to single drug or lower antibiotics based on the culture report. Meropenem and vancomycin are reserved as second line drugs for cases where the patient is extremely sick, or not responding/ deteriorating/culture reports show resistance to first line drugs. Ertapenem although showed good sensitivity results, was not used because of unknown safety profile in neonates. But it holds promise in future once the safety profile in neonates is established. Since majority of candida species were sensitive to fluconazole, use of fluconazole as first line antifungal in appropriate clinical settings seemed judicious in present setup.

CONCLUSION

Neonatal sepsis is an important cause of neonatal deaths. Management depends upon the microbes involved and their antimicrobial susceptibility pattern. In present study, *K. pneumoniae* and CONS were most important pathogens. Both these pathogens were multidrug resistant. Non albicans Candida were responsible for majority of fungal sepsis.

WHO guidelines suggest use of penicillin and gentamicin for neonatal sepsis. But in present study, they were not found useful, instead amikacin and netilmicin were beneficial. Authors recommend regular monitoring of antimicrobial susceptibility pattern followed by necessary changes in antibiotic policy for reasonable empirical therapy. High isolation rates of CONS suggest improvement of blood culture sample collection methods and provision of trained phlebotomists for the same. Hospital administration should provide enough support for the same.

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