Antimicrobial Resistance Situation in SCANU of Faridpur Medical College Hospital, a Tertiary Level Hospital in Bangladesh

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Abstract:
Considering the high risk of neonatal sepsis in low and middle-income countries, empirical antibiotic therapy is commonly employed in most cases to clinically suspected septic neonates without the aid of culture and antibiotic sensitivity report. Increasing antimicrobial resistance is posing a threat to these practices and urges the obligation for understanding the causative organisms and their changing resistance pattern at the local level. Aim of this study was to identify the pathogens responsible for neonatal sepsis and understand their current antimicrobial resistance pattern. This prospective study was undertaken in the Special Care Newborn Unit of the Faridpur Medical College Hospital from October 2017 to November 2018. Venous blood culture and antimicrobial susceptibility of 56 suspected neonatal sepsis cases were studied. Among the 56 clinically suspected cases 86% had culture positive isolates in the specimen. Predominant isolates were *Klebsiella* (42%) and coagulase-negative staphylococci (25%). Of all the identified bacteria, 88% were resistant to ≥ 3 classes of antibiotics. Eighty-five percent of *Klebsiella* isolates were found to be carbapenem-resistant along with 100% of *E. coli* isolates and 95% of *Klebsiella* isolates had possible extended spectrum β-lactamase production. Seventy-five percent of *Acinetobacter* isolates were multidrug-resistant and 100% of coagulase-negative staphylococci were methicillin resistant. The array of causative organisms and their increasing resistance to commonly practiced antibiotics are alarming. It is urgent to develop strategies focusing on all healthcare levels to cease the spread of antimicrobial resistance in an effort to reduce the burden of neonatal sepsis.

Key words: Neonatal Sepsis, Antimicrobial Resistance.

Introduction:
Annually, an estimated 2.7 million neonates (0-28 days of life) die worldwide, and approximately 98% of these deaths occur in developing countries1-3. Among the various causes, neonatal sepsis is one of the main reasons for neonatal mortality and morbidity. Globally it accounts for nearly 2.1 million deaths every year2.

However, resource-poor countries such as Sub-Saharan and South Asian countries bear the greatest burden of it2-3. Roughly 1 million deaths each year in the developing countries are attributable to infections acquired in neonatal period4-6-7.

Currently, in Bangladesh, the majority of births take place at home, and many of the newborns with sepsis do not come to medical attention8. In addition, most districts and community hospitals do not have facilities to perform blood cultures, and the diagnosis of neonatal sepsis is mostly done clinically9. As a result, estimating the burden of neonatal sepsis in Bangladesh is challenging. However, one recent population-based surveillance found 14.5% of newborns had infections in their first 9 days of life10.

With the advancement of the Neonatal Intensive Care Unit (NICU) and Special Care Newborn Unit (SCANU), the quality of care and the survival of the preterm, low birth weight infants have increased11. However, it has created its own sets of risks. Bacterial sepsis is a common scenario in NICU and SCANU particularly in preterm, low birth weight infants and the overall death rates from neonatal sepsis range from 2% to as high as 50% in some cases12-18.
Considering the significant risk of infection in the newborn, empiric antimicrobial therapy is commonly employed, but the treatment of neonatal sepsis is threatened by the steady increase in the prevalence of antimicrobial resistance (AMR). Although WHO recommends ampicillin and gentamicin combination for the treatment of neonatal sepsis, hospital-based data show an alarming rate of resistance to these drugs among common pathogens causing neonatal sepsis. On the other hand, the spectrum of organisms responsible for sepsis changes with geographic regions. To formulate strategies to treat neonatal sepsis and halt the spread of antimicrobial resistance, region and center-specific knowledge about the common causative organisms, their changing patterns and antibiotic resistance status are essential.

This study was undertaken to determine the current distribution of infecting pathogens along with antibiotic sensitivity and resistance profile in a cohort of neonates diagnosed with neonatal sepsis at Faridpur Medical College Hospital, Bangladesh.

Materials and methods:

This prospective study was undertaken in the Special Care Newborn Unit (SCANU) of the Faridpur Medical College Hospital (FMCH), Bangladesh from October 2017 to November 2018. During this period, 56 suspected cases of neonatal sepsis were studied. The neonates were selected as septicemic based on clinical evaluations.

Venous blood was obtained from the selected patients with proper aseptic precautions and sent for culture and antibiotic susceptibility testing in the clinical microbiology laboratory of the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b). Blood culture bottles were placed in the BacT/ALERT machine. If the results were positive, then the broth was sub-cultured. Isolated colonies were identified and antibacterial susceptibility was tested using the VITEK-2 system.

Descriptive analysis was performed using STATA 15 (StataCorp. College Station, TX).

MDR (Multi Drug Resistant) was defined as resistant to three or more classes of antibiotics. ESBL (Extended Spectrum Beta-Lactamase) was defined only based on resistance to cefazidine or ceftriaxone, confirmatory tests using tests in combination with clavulanic acid was needed but not done.

Result:

The predominant isolates were Klebsiella 20 (41.7%) and coagulase-negative staphylococci (CoNS) 12 (25.0%). The isolates of Acinetobacter 4 (8.3%), E. coli 4 (8.3%), gram-positive bacillus (GPB) 3 (6.3%), Enterobacter 2 (4.2%), Enterococcus 1 (2.1%), Pseudomonas 1 (2.1%) and Streptococcus 1 (2.1%) were also observed but in lesser proportions (Table I).

Table I: Identified bacterial* isolates (n=48)

| Name of the organism | Number of isolates | Percentage |
|----------------------|--------------------|------------|
| Klebsiella           | 20                 | 42%        |
| CoNS                 | 12                 | 25%        |
| Acinetobacter        | 4                  | 8%         |
| E. coli              | 4                  | 8%         |
| GPB                  | 3                  | 6%         |
| Enterobacter         | 2                  | 4%         |
| Enterococcus         | 1                  | 2%         |
| Pseudomonas          | 1                  | 2%         |
| Streptococcus        | 1                  | 2%         |

*CoNS: coagulase-negative staphylococci

GPB: Gram-positive bacillus

*Level of taxonomic classification varies by isolates. Some isolates were grouped under CoNS for convenience.

Among the identified bacterial isolates, 42 (87.5%) were resistant to ≥ 3 classes of antibiotics. Among them 19 (95%) Klebsiella isolates were resistant to ≥ 3 classes of antibiotics. All isolates of CoNS, Acinetobacter, E. coli, Enterococcus, and Streptococcus were resistant to ≥ 3 classes of antibiotics as well. All the GPB isolates were found to be sensitive to all of the antibiotic classes (Table II).

Table II: Number of antibiotic classes with resistance

| Name of the organism | Number of antibiotic classes 0 | 1 | 2 | 3 | Total |
|----------------------|-------------------------------|---|---|---|-------|
| Klebsiella           | 0 (0%)                        | 1 (5%) | 0 (0%) | 19 (95%) | 20     |
| CoNS                 | 0 (0%)                        | 0 (0%) | 0 (0%) | 12 (100%) | 12     |
| Acinetobacter        | 0 (0%)                        | 0 (0%) | 0 (0%) | 4 (100%) | 4      |
| E. coli              | 0 (0%)                        | 0 (0%) | 0 (0%) | 4 (100%) | 4      |
| GPB                  | 3 (100%)                      | 0 (0%) | 0 (0%) | 0 (0%) | 3      |
| Enterobacter         | 0 (0%)                        | 1 (50%) | 0 (0%) | 1 (100%) | 2      |
| Enterococcus         | 0 (0%)                        | 0 (0%) | 0 (0%) | 1 (100%) | 1      |
| Pseudomonas          | 0 (0%)                        | 0 (0%) | 0 (0%) | 1 (100%) | 1      |
| Streptococcus        | 0 (0%)                        | 0 (0%) | 1 (100%) | 0 (0%) | 1      |
| Total                | 3 (6%)                        | 2 (4%) | 1 (2%) | 42 (88%) | 48     |
Among the Enterobacteriaceae isolates, carbapenem resistance was found to some extents in all isolates; *Klebsiella* 17 (85%), *Enterobacter* 1 (50%), and E. coli 1 (25%). Resistance to colistin was found only in *Klebsiella* 6 (30%), and all which were also resistant to carbapenem. Based on resistance to ceftazidime or ceftriaxone, all of the *E. coli* isolates 4 (100%) were found to be producing extended spectrum b-lactamase (ESBL). Nineteen (95%) *Klebsiella* isolates and 1 (50%) *Enterobacter* isolate had possible ESBL production (Table III).

**Table III: AMR among Enterobacteriaceae isolates**

| Antimicrobial agents and ESBL production | Klebsiella (N=20) | Enterobacter (N=2) | E. coli (N=4) |
|----------------------------------------|------------------|-------------------|--------------|
| Carbapenem                             | 17 (85%)         | 1 (50%)           | 1 (25%)      |
| Colistin                               | 6* (30%)         | 0 (0%)            | 0 (0%)       |
| Possible ESBL production**             | 19 (95%)         | 1 (50%)           | 4 (100%)     |

*All were also carbapenem-resistant

**Only based on resistance to ceftazidime or ceftriaxone. Most were resistant to both.

When carbapenem resistant *Klebsiella* isolates were plotted over the study time, one or more isolates resistant to carbapenem were found in 9 occasions whereas isolates not resistant to carbapenem were found in 3 occasions. Most of the carbapenem-resistant *Klebsiella* isolates were found between the 28th to 37th weeks of the study period (Figure I).

Over the study period, the two most common *Klebsiella* antibiogram profiles were also observed. Between the 28th to 34th weeks, a group of *Klebsiella* was found to be resistant to all antibiotic groups (Figure II).

**Figure II: Two most common Klebsiella antibiogram profiles* over time**

*Based on antibiogram using sensitivity to amikacin, ampicillin, cefepime, ceftazidime, ceftriaxone, cefuroxime, ciprofloxacin, colistin, cotrimoxazole, gentamicin, imipenem, meropenem, and piperacillin/tazobactam. All other antibiogram profiles were unique to one isolate or shared by one other isolate.

Among the 4 *Acinetobacter* isolates, 3 (75%) were found to be multidrug-resistant (MDR) according to the CDC definition\textsuperscript{30}. Among the others, 1 (25%) was colistin resistant and 1 (25%) was tigecycline resistant (Table V).

**Table V: AMR among Acinetobacter isolates**

| Antibiotic resistance | Number (N=4) | Percentage |
|-----------------------|--------------|------------|
| MDR                   | 3            | 75%        |
| Colistin-resistant    | 1            | 25%        |
| Tigecycline-resistant | 1            | 25%        |

During the study period, MDR *Acinetobacter* isolates were found roughly at the beginning, middle, and ending of the study (Figure III).
Among the other bacteria, 12 (100%) coagulase-negative staphylococci were methicillin-resistant and none of the (0%) Enterococcus isolates was resistant to vancomycin (VRE).

Discussion:

Among the 56 suspected neonatal sepsis blood specimens that we cultured, 86% of them had various types of bacterial isolates. Only 14% of the clinically suspected septic neonates had no bacteria detected in their blood culture. Although the majority of neonates with clinically diagnosed sepsis had proven isolated organisms in their blood, all of them, including those without detectable bacterial isolates received empiric antibiotic therapy.

Of the different isolated organisms, Klebsiella, CoNS, Acinetobacter, and E. coli were found to be the most dominant and contributed to neonatal sepsis in more than 75% cases collectively. Klebsiella alone was the responsible organism for approximately 40% of the septic neonates.

Many studies reported that CoNS are the most common organisms associated with neonatal sepsis, but those were found in high-income countries and was mostly in late onset neonatal sepsis 17,31-35. However, we found CoNS (24%) to be the second most common organisms and Klebsiella to be the commonest. Our findings coincide with studies done by Genatra et al., Karunasekera et al., Tallur et al., Teresa et al. 36-38 that looked into the neonatal infection pattern in low- and middle-income countries; where they found Klebsiella as the predominant pathogen responsible for nearly 25% of all cases of neonatal sepsis 11.

In our study, we found only 1 isolate of Pseudomonas and 1 of Streptococcus. These findings differ from prior studies where they found a higher association of these organisms with neonatal sepsis 5,11,13,23,39. This may be due to geographic region-specific dominance of specific organisms or simply may be due to difference in sampling approach. However, some studies suggest that despite a high rate of colonization, the attack rate of Staphylococcus aureus infections are relatively as low as 2% 16,40.

We found no case of sepsis with group B streptococcus which is more common in early onset neonatal sepsis in high income countries but not in low- and middle-income countries 41.

Colonization and subsequent infection with strains of antibiotic resistant bacteria has become a major concern in hospitalized neonates with a greater risk in South-East Asian countries, where the rate of antibiotic resistance is higher 25.

In our study, among the Enterobacteriaceae isolates, Klebsiella was the commonest. Increasing antibiotic resistance among Enterobacteriaceae isolates has raised a major concern as sepsis with these organisms are associated with considerable morbidity and mortality of the neonates 16,18,42,43. Among the members of the Enterobacteriaceae family that we found, resistance to carbapenem was found in 85% of Klebsiella isolates, 50% of Enterobacter isolates, and 25% of E. coli isolates. Moreover, 30% of the Klebsiella isolates were colistin-resistant, all of which were also resistant to carbapenem. Nearly all of the E. coli and Klebsiella isolates and half of the Enterobacter isolates had possible ESBL production. Such a picture of resistance even to newer generations of antibiotics is a great threat to the upcoming future. Recent studies agree that members of Enterobacteriaceae family are most often resistant to at least one of the classical parenteral antibiotics used in neonates 16,44,45.

All of the CoNS isolates that we found were resistant to ≥ 3 antibiotic classes. It is an alarming situation as they are resistant to the routine antibiotics that are used to treat neonates and often require vancomycin or other antibiotics for adequate therapy. This similar resistance pattern is being found in a growing number of studies 17,20.

Infection with such organisms presents a serious challenge to clinicians as the antibiotic choice for treating such infection is limited. In such cases, associated mortality has also been found to be higher 17.
Of the 4 Acinetobacter isolates that we found, 75% were MDR. One of the isolates (25%) was colistin-resistant and another one (25%) was tigecycline-resistant. Studies done in other parts of Asia have found 15% to as high as 22% of all tested antibiotic-resistant isolates of Acinetobacter.

Also, we found such resistant isolates of Klebsiella and Acinetobacter were almost evenly at different times throughout our study period.

Over time, the evolution of hyper virulent clones of bacteria causing neonatal sepsis will become an increasing problem unless controlled effectively.

With such an antibiotic resistance pattern and the emergence of MDR bacteria will pose a great challenge to the recommended empiric use of ampicillin and gentamycin combination for the treatment of neonatal sepsis.

Conclusion:

Increasing antimicrobial resistant pattern is often leaving us with a few antibiotics as a safe resort for treating highly-resistant virulent organisms. Strategies and guidelines at the local level need to be formulated to halt the global spread of antimicrobial resistance.

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