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Mohammed A. Kubas

Massoud K. Rassam

Amaal A. Alsayani

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ORIGINAL ARTICLE

Antibiogram Development for Neonatal Intensive Care Unit in a Referral Hospital in Sana'a City, Yemen

Mohammed A. Kubas^{a,b,c,*}, Massoud K. Rassam^d, Amaal A. Alsayani^b

^a Clinical Pharmacy Department, School of Pharmacy & Medical Science (SPMS), Lebanese International University (LIU), Sana'a, Yemen

^b Clinical Pharmacy & Pharmacy Practice Department, Faculty of Pharmacy, University of Science & Technology (UST), Sana'a, Yemen

^c Clinical Pharmacy Department, University of Science & Technology Hospital (USTH), Sana'a, Yemen

^d Clinical Pharmacy Department, Faculty of Pharmacy, Emirates International University (EIU), Sana'a, Yemen

ABSTRACT

Neonates have an immature immune system, making them highly susceptible to infections. Treatment failure in newborns may result from antibiotic resistance, a significant challenge in the management of infectious illnesses worldwide. So the main objective of this research was to develop a newborn-specific antibiogram at the University of Science and Technology hospital (USTH) in Sana'a, Yemen. A retrospective analysis was conducted using microbiological data from the USTH database during the period from January 2021 to March 2022, antibiogram was generated and analyzed using the WHONET application. Gram-positive bacteria were the most frequently isolated pathogens in neonates, followed by gram-negative bacteria. The most frequently isolated gram-positive bacteria were *Streptococcus* spp. and coagulase-negative staphylococci, while the most frequently isolated gram-negative bacteria were *Burkholderia cepacia* and *Klebsiella* spp. The most sensitive antibiotics for gram-positive bacteria were vancomycin and linezolid, whereas carbapenem, colistin, and polymyxin B were the most sensitive antibiotics for gram-negative bacteria. High levels of resistance were noted across both bacterial groups, emphasizing the urgent need for updated local antibiograms to guide empirical therapy. These findings can directly inform antibiotic stewardship efforts and contribute to the development of evidence-based neonatal care guidelines in resource-limited settings like Yemen.

Keywords: Neonates, Antibiogram, USTH, Gram-positive bacteria, Gram-negative bacteria, Antibiotics

1. Introduction

Neonates are susceptible to infections caused by various pathogenic microorganisms due to a lack of functional innate or adaptive immunity [1], which affects them from birth to four weeks of age [2]. Bacterial infections that affect neonates range from mild to life-threatening and include bloodstream infections (BSIs), urinary tract infections (UTIs), meningitis, endocarditis, osteomyelitis, pyogenic arthritis, ventilator-associated pneumonia, peritonitis, conjunctivitis, and skin abscesses [3]. Based on the

timing, neonatal infections can develop early (within the first 7 days of life) or late (after 7 days of delivery) [4]. The immune systems of neonates are rapidly developing, and their ability to combat infections may vary depending on their age [4].

Bacterial resistance to antibiotics typically results from genetic changes induced by the overuse of antibiotics [5, 6]. Antimicrobial resistance (AMR) can also develop as a result of improper antibiotic use. This resistance continues to be a global crisis, with over 2.8 million infections and over 35,000 deaths reported annually from 2012 through 2017 [7]. It

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* Corresponding author.

E-mail address: drkubasm@gmail.com (M. A. Kubas).

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is concerning that neonatal infections are becoming more resistant to antibiotics used for treating bacterial infections like sepsis and UTIs [8]. According to the World Health Organization (WHO) [9], 2–70% of national data show resistance to third-generation cephalosporins in *Escherichia coli* and 8–77% in *Klebsiella pneumoniae*. In addition, 14–71% of *E. coli* isolates were found to be resistant to fluoroquinolones, 12–80% of *Staphylococcus aureus* were resistant to methicillin, and 3–16% of *Streptococcus pneumoniae* isolates were resistant to penicillin [9].

These national data on gram-negative bacteria align with findings from sub-Saharan Africa [10–17], showing that *E. coli* and *K. pneumoniae* are generally less susceptible to penicillins, cephalosporins, fluoroquinolones, and trimethoprim/sulfamethoxazole (TMP/SMX). However, these pathogens remain highly susceptible to amikacin and carbapenems. Between 2006 and 2013, *E. coli* and *Acinetobacter* species were the most frequently isolated gram-negative bacteria from inpatients admitted to the University of Science and Technology Hospital (USTH) departments in Sana'a, Yemen, followed by *Klebsiella* species and *Pseudomonas aeruginosa* [18]. *Acinetobacter* species was the most resistant to routinely used antibiotics, with polymyxin B being the only effective antibiotic against this bacterium [18]. In Aden, south of Yemen, *Staphylococcus* species were found to be the most common pathogenic bacteria in multiple isolates, followed by *E. coli*, *Pseudomonas* species and *K. pneumoniae*, with resistance being highest against TMP/SMX, followed by amoxicillin plus clavulanate and cephalosporins [19].

Antimicrobial susceptibility patterns of pathogens vary geographically and depend on local pathogens and antibiotic use. These patterns also change from time to time, even within the same practice region [20]. Antibiograms play a crucial role in identifying patterns that differ or change in order to select the right antibiotics for practical usage and epidemiological surveillance [21]. However, many low- and middle-income countries, including Yemen, lack sufficient neonatal-specific data on antimicrobial resistance. Despite growing concerns about antimicrobial resistance, there is a notable lack of published data on neonatal-specific antibiograms in Yemen [18]. This gap limits clinicians' ability to make informed empirical antibiotic choices in NICUs across the country. Therefore, this study aimed to develop an antibiogram specific for neonates at the Neonatal Intensive Care Unit (NICU) of the USTH in Sana'a, Yemen.

2. Methods

2.1. Study design, setting, and population

A retrospective, cross-sectional study was conducted at the NICU of the USTH in Sana'a, Yemen, from January 2021 to March 2022. The USTH is a 165-bed private teaching hospital that serves as a major referral hospital and provides clinical training for physicians, nurses, and pharmacists. Data about antibiotic susceptibility were extracted from the records of neonates admitted to the NICU.

2.2. Data collection and analysis

Data of all isolates, their antibiotic susceptibility, and the gender of neonates were retrieved from the electronic system of the NICU. Then, WHONET 5.6 software (<https://whonet.org/software.html>) was used to enter and analyze susceptibility data.

2.3. Antibiogram development

For each bacterial species, susceptibility percentages were calculated from the pooled data using the WHONET 5.6 software. The auto-generated susceptibilities were reviewed by the research team to ensure accuracy. Bacterial species with fewer than 30 isolates were excluded from analysis. However, clinically relevant organisms were included in the analysis based on the head of the department of neonatology's department advice, even if having fewer than 30 isolates.

The list of antibiotics included in the antibiogram was restricted to those antibiotics that are regularly available at the USTH. A single antibiogram was developed for pediatric physicians to incorporate precise susceptibility percentage data for commonly used antibiotics, with symbols representing susceptibility ("R" for resistant, "I" for intermediate, and "S" for sensitive). In this antibiogram, the bacterial species were classified as resistant if 0–59.9% susceptible, intermediate if 60–79.9% susceptible, and sensitive if 80–100% susceptible [22]. In the case of intrinsic resistance (resistance that is naturally present in the microorganism) of an organism to an antibiotic, this was labeled as "R" instead of providing the percentage of susceptibility.

2.4. Ethical approval and consent

Since this was a retrospective investigation using de-identified specimens, no consent form was needed,

and the study was approved by the administration at USTH and EIU (2022/002/FREC/EIU).

3. Result

3.1. Gender of neonates with confirmed infections and sources of specimens

A total of 324 cultures were screened from 257 neonates between January 2021 and March 2022. Of these, 176 (54.3%) showed no growth, while 148 (45.7%) were positive for bacterial isolates from 133 patients (72.9% males and 27.1% females), as shown in Table 1. Blood was the most common source of isolation, accounting for 89.9% of cases, while other sources, such as urine, swabs, and sputum, represented the remaining specimens.

Table 1. Gender-distribution of neonates with culture-proven infection admitted to the NICU of the USTH in Sana'a, Yemen (2021–2022)*.

Gender	N(%)
Male	97 (72.9)
Female	36 (27.1)

* The total number of neonates with culture-proven infection was 133. NICU, Neonatal Intensive Care Unit; USTH, University of Science and Technology Hospital.

3.2. Antibigram for gram-positive bacteria

Among the 94 gram-positive isolates, coagulase-negative staphylococci (CoNS) were the most frequent clinical isolates (73.4%), followed by *Streptococcus* species (9.7%), *S. aureus* and *Enterococcus*

species (6.3% each), and *Staphylococcus hominis* (4.3%). CoNS had maintained susceptibility to vancomycin, linezolid, while other antibiotics, such as cephalosporins, showed high resistance against most gram-positive bacteria Table 2.

3.3. Antibigram for gram-negative bacteria

Among the 44 gram-negative bacterial isolates, *Klebsiella* spp. (12 isolates, 8.7%) and *Burkholderia cepacia* (9 isolates, 6.5%) were the most frequently isolated gram-negative bacteria. *Klebsiella* spp. exhibited high resistance to most antibiotics, except for imipenem, polymyxin B, and colistin, whereas *Burkholderia cepacia* showed susceptibility to meropenem Table 3.

Susceptibility rates for *Klebsiella* spp. were as follows: piperacillin/tazobactam (57.1%), cefotaxime (25%), and gentamicin (36.4%). The high resistance to third-generation cephalosporin indicated high rates of beta-lactamase production, including extended-spectrum beta-lactamases (ESBL).

4. Discussion

The isolated bacteria from USTH at the NICU were examined, and the following were the main findings: (1) gram-positive bacteria (i.e., coagulase-negative staphylococci) were the most isolated bacteria in neonatal patients. (2) Linezolid and Vancomycin were the most sensitive antibiotics for gram-positive bacteria; and (3) The majority of isolated gram-negative bacteria were resistant to most cephalosporin generations and primarily responsive

Table 2. Antibigram for Gram-positive bacteria at the NICU of the USTH (2021–2022).

Gram-positive bacterial isolates (N = 94) n (%)	Antibiotic susceptibility [# of sensitive antibiotics/ total # of antibiotics tested] (%) ^a														
	Amikacin	Azithromycin	Cefepime	Cefoperazone	Cefotaxime	Ceftazidime	Clindamycin	Colistin	Ampicillin	Ampicillin/sulbactam	Gentamicin	Imipenem	Linezolid	Penicillin G	Piperacillin/tazobactam
<i>Staphylococcus aureus</i> 6 (6.3)	[4/5] (80)	[1/6] (16.7)					[4/6] (66.7)				[4/6] (66.7)		[6/6] (100)		[6/6] (100)
Coagulase-negative staphylococci 69 (73.4)	[48/58] (82.8)	[10/54] (18.5)	[0/1] (0)				[44/66] (66.7)		[0/1] (0)		[39/65] (60)		[65/65] (100)	[1/7] (14.3)	[67/67] (100)
<i>Staphylococcus hominis</i> 4 (4.3)							[3/4] (25)				[2/4] (50)		[4/4] (100)		[4/4] (100)
<i>Streptococcus</i> species 9 (9.7)		[5/6] (83.3)					[8/9] (88.9)		[5/7] (71.4)				[7/7] (100)	[7/9] (77.8)	[9/9] (100)
<i>Enterococcus</i> species 6 (6.3)									[2/5] (40)				[6/6] (100)	[2/6] (33.3)	[6/6] (100)
	≥ 80% of isolates susceptible			60–79% of isolates susceptible			≤ 59% of isolates susceptible			Not determined					

^aNote: percentages are calculated based on the number of antibiotics tested not based on total bacteria isolated; e.g. in staphylococcus, the total isolated is 6 but amikacin only tested 5 times out of 6. Therefore, susceptibility calculated based on tested amikacin (i.e. 5) and its susceptibility equal 80%, meaning that out of 5 tested isolates, only 4 were sensitive to amikacin.

Table 3. Antibigram for Gram-negative bacteria at the NICU of the USTH (2021–2022).

Gram-negative bacterial isolates (N = 44) n (%)	Antibiotic susceptibility [# of sensitive antibiotics/ total # of antibiotics tested] (%) ^a															
	Amikacin	Azithromycin	Cefepime	Cefoperazone	Cefotaxime	Ceftazidime	Clindamycin	Colistin	Ampicillin	Ampicillin/sulbactam	Gentamicin	Imipenem	Linezolid	Penicillin G	Piperacillin/tazobactam	Vancomycin
<i>Acinetobacter</i> species 3 (6.8)	[1/3] (33.3)		[0/2] (0)		[0/3] (0)	[0/3] (0)		[2/2] (100)			[0/2] (0)	[3/3] (100)			[3/3] (100)	[1/1] (100)
<i>Escherichia coli</i> 6 (13.6)	[5/5] (100)		[1/6] (16.7)	[0/3] (0)	[1/3] (33.3)	[1/6] (16.7)			[0/4] (0)	[0/1] (0)	[6/6] (100)	[5/5] (100)			[2/4] (50)	[6/6] (100)
<i>Enterobacter</i> species 3 (6.8)			[0/2] (0)		[1/2] (50)	[0/2] (0)			[0/2] (0)		[1/2] (50)	[2/3] (66.7)		[1/1] (100)	[1/2] (50)	[1/2] (50)
<i>Klebsiella</i> species 12 (27.3)	[7/12] (58.3)		[1/12] (8.3)	[0/6] (0)	[1/4] (25)	[0/12] (0)		[1/1] (100)	[0/7] (0)	[0/3] (0)	[4/11] (36.4)	[6/7] (85.7)			[4/7] (57.1)	[1/1] (100)
<i>Klebsiella pneumoniae</i> 5 (11.4)	[5/5] (100)		[1/5] (20)			[1/5] (20)			[0/5] (0)	[1/5] (20)	[4/5] (80)					[4/5] (80)
<i>Burkholderia cepacia</i> 9 (20.5)						[4/9] (44.4)										[8/8] (100)
<i>Pseudomonas</i> species 6 (13.6)	[3/6] (50)		[1/5] (20)	[0/1] (0)		[1/6] (16.7)					[3/6] (50)	[6/6] (100)			[5/6] (83.3)	[6/6] (100)
≥ 80% of isolates susceptible 60–79% of isolates susceptible ≤ 59% of isolates susceptible Not determined																

^aNote: percentages are calculated based on the number of antibiotics tested not based on total bacteria isolated; e.g. in *Acinetobacter*, amikacin shows 33.3% susceptibility, meaning that out of 3 tested isolates, only 1 were sensitive to amikacin.

to carbapenem antibiotics. Community features, non-adherence to infection control standards, and local antibiotic prescribing protocols are some causes of high resistance rates for antibiotics.

This study found that 89.9% of neonates had blood culture proven bacteria, making it the most isolated source. This finding was consistent with previous studies conducted in South East Nigeria and South Africa, which reported blood culture prevalence rates of 96.7% and 55.2%, respectively [20, 23]. In contrast to Kumar's findings, we found that 94 of these isolates are gram-positive and 44 of them are gram-negative isolates [24].

According to this study, coagulase-negative staphylococci isolates accounted for 69 (50%), which represented the most often isolated gram-positive bacteria at the USTH hospital. The higher proportion of gram-positive bacteria in this study is similar to studies done in South East Nigeria, South Africa, Nepal, and North Eastern Nigeria [20, 23–25]. Since our investigation was retrospective, we were unable to determine whether the high percentage of gram-positive bacteria in our institution was due to contamination, different locations, people, or clinical situations.

The most susceptible drugs for gram-positive bacteria were vancomycin and linezolid, which is in line with other studies [20, 25]. In another study, they had demonstrated that Gentamicin was the most sensitive antibiotic for *S. aureus* [25], while in our findings, we found that gentamicin has intermediate sensitivity, 66.7%.

The present study showed that *Klebsiella* spp was the most isolated gram-negative bacteria, 8.7%. This

result ties well with the previous study [23–25, 27], followed by *Burkholderia cepacia*, 6.5%. According to prior research done in South East Nigeria and Zambia [20, 22], the gram-negative isolates had significant levels of resistance to cephalosporins and high sensitivity to carbapenem, which was in line with our finding; this could be an indication of ESBL for these isolates. However, another study had demonstrated that gram-negative isolates had a high level of sensitivity to cephalosporins [26].

There were certain restrictions on this investigation. Initially, the data used in this retrospective investigation were gathered from laboratory records, which did not include details regarding the neonates' date of hospitalization, clinical status, or course of therapy. As a result, we were unable to categorize infections as having been acquired from the hospital or the community. In a similar vein, we were unable to identify primary or secondary antibiotic resistance. Furthermore, this study did not contain information on the neonates' clinical characteristics or treatment outcomes. Additionally, our study was limited to a single hospital, so the patterns of antibiotic resistance may not be representative of the entire nation's circumstances. The findings of this study highlight the urgent need for continuous monitoring of antimicrobial resistance patterns in neonatal populations. While this retrospective analysis offers valuable baseline data, prospective multicenter studies are essential to capture evolving trends over time and to differentiate between community-acquired and hospital-acquired infections. Additionally, the development of a neonatal-specific antibiogram provides a practical foundation for implementing evidence-based

antibiotic stewardship interventions in NICUs. These findings can support local healthcare providers in refining empirical treatment guidelines, optimizing antimicrobial use, and ultimately improving neonatal outcomes in Yemen and similar resource-limited settings.

5. Conclusion

Neonates continue to exhibit high levels of resistance to both gram-positive and gram-negative bacteria. Gram-positive bacteria were most sensitive to vancomycin and linezolid, while gram-negative bacteria were most sensitive to carbapenem and colistin antibiotics. A national-level antibiogram for neonates is essential, given the high resistance rates observed. Antibiotic stewardship programs should be implemented to reduce unnecessary antibiotic use.

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Conflict of interest

The authors declare no conflict of interest associated with this article.

Ethical approval

Ethical review and approval were waived for this study, due to this observational study, and the specimen was de-identified, and the Faculty Research Ethical Committee gave us administrative approval with this number (2022/002/FREC/EIU).

Data availability

The data that support the findings of this study are available on request from the corresponding author.

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None

Author contributions

The concept was conceived of by Kubas, who also created the research methodology. The results portion of the study was analyzed by Rassam. Alsayani helped write the manuscript. Every author participated in the process of revising and rewriting the manuscript's last version.

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