Antibiogram Development for Neonatal Intensive Care Unit at Referral Healthcare Center in Sana'a City, Yemen

Submitted By

Massoud Khalid Rassam
Aimen Ahmed Qasem Alhebshi
Noman Mohammed Abdulmajeed
Mohammed Hassan Al Nuzaili
Majde Mohammed Saif
Ala'a Fuod Alsalowi
Lool Abdulrahman Amr
Yusra Ahmed Al Mashmali
Ghada Adnan Salah
Amr Abdullah Nasher
Khalid Ali Alodini

Supervised by

Associate. Prof Dr \ Mokhtar Al-Ghorafi
Dr \ Mohammed Abdullah Ali Kubas

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ABBREVIATION

AB	Antibiotic
AMR	Antimicrobial resistance
BSI	Bloodstream infections
CDC	Centers for Disease Control and Prevention
CoNS	Coagulase negative staphylococcui
CPE	Carbapenemase-producing Enterobacteriaceae.
CRE	Carbapenem-Resistant Enterobacteriaceae
E.coli	Escherichia coli
ESBL	Extand specteram beta lactamiases
G +ve	Gram positive
G -ve	Gram negative
GBS	Group B streptococcus
GN	Gram negative
GP	Gram positive
HAIs	Healthcare associated infections
HIV	Human immunodeficiency virus
HSV	Herpes simplex virus
I	Intermediate
ICU	Intensive care unite
ICUHTLV	Human T-lymphotrophic virus type 1
MRSA	Methicillin-resistant Staphylococcus aureus
NICU	Neonates intensive critical care sensitive
No	Number of

P. aeruginosa	Pseudomonas aeruginosa
PBP	Penicillin-Binding Protein
R	Resistance
S	Sensitive
S. aureus	Staphylococus aureas
SCC	Staphylococcal cassette chromosome
TMP/SMX	Trimethoprim/sulfamethoxazole
USTH	University science and teqnology hospital
UTIs	Urinary Tract Infections
VRSA	Vancomycin Resistance Staphylococus Aureus
WHO	World Health Organization

ABSTRACT

Background

The neonate immunity is very low and infections among them could lead to life threating consequences. Antibiotic resistance is considered one of the most challenging area in the treatment of infectious diseases worldwide and could lead to treatment failure among neonate.

Objective

The overall aim of this study was to develop antibiogram that specific for neonate at USTH, Sana'a, Yemen.

Methodology

This is retrospective study, the data was collected from the USTH database and WOHNET program was used for analysis and development of USTH antibiogram

Result

The G +ve was the most common type of bacteria encounter among neonate, followed by G –ve bacteria. From G +ve bacteria, staphylococcus coagulase negative and Streptococcus spp were the most common isolated bacteria, and klebsiella spp and Burkholderia cepacia were the most isolated among G-ve bacteria. The most sensitives antibiotic for G+ve bacteria was vancomycin and linezolid, whereas, carbapenem, colistin, and polymyxin B were the most sensitive antibiotics for G-ve bacteria.

Conclusion

The resistance for antimicrobial agent was high among neonate patient ether for G+ve positive or G-ve bacteria.

Chapter One

Introduction

INTRODUCTION

Neonates or newborns can be defined as babies from the time of delivery up to four weeks old.[1] Neonates possess an incompetent innate or/and adaptive immunity, which make them more susceptible and less able to compete with infections caused by numerous pathogenic microorganisms.[2]

Bacterial infections in newborns can range from mild to severe and life-threating infections, include Bloodstream infections (BSIs) are the most common. They can occur in isolation or in association with urinary tract infections (UTIs) and meningitis. Endocarditis, osteomyelitis, pyogenic arthritis, ventilator associated pneumonia , peritonitis, conjunctivitis, and skin abscesses are important, less common HAIs[3].

Based on the age of neonates when contracting the infection, the infection can be categorized into two groups: early (during the first 7 days of life) and late (after 7 days from delivery) onset.[4]

This is because the immune system of neonatesmis rapidly developing as they are growing, thus each stage of age may possess different level of potency to fight infections.[4] This fact might explain the reasons behind observed variation of causative bacteria according to baby's age group.[2] Resistance of bacteria to antibiotics is usually caused by genetic modifications as a result of the irrational use of antibiotics.[5,6]

He rise in antimicrobial resistance (AMR) continues to be a global crisis. Collectively, antimicrobial-resistant pathogens caused more than 2.8 million infections and over 35,000 deaths annually from 2012 through 2017, according to the 2019 Centers for Disease Control and Prevention (CDC) Antibiotic Resistance Threats in the United States Report.[7]

Multiple factors transcending disciplines contribute to the development of AMR, with inappropriate use of antibiotics regarded as a major contributing factor according to the report by the WHO Global Action Plan on antimicrobial resistance.[8] The increase level of resistance against antibiotic drugs used to treat bacterial infections associated with sepsis and UTI in neonates is very alarming worldwide.[9]

On national data of ABR reported by the World Health Organization (WHO) indicates Escherichia coli and Klebsiella pneumoniae resistance to 3rd generation cephalosporin at 2–70% and 8–77%, respectively.[10]

E. coli resistance to fluoroquinolones is reported at 14–71% [10]. Methicillin-resistant Staphylococcus aureus (MRSA) is reported to make up 12–80% of S. aureus isolates while Streptococcus pneumoniae resistance to penicillin is at 3–16%. [10] Available literature from SSA supports this national data. E. coli and K. pneumoniae generally have low susceptibility to penicillin, cephalosporin, fluoroquinolones, and trimethoprim/sulfamethoxazole (TMP/SMX) while maintaining high susceptibility to carbapenems and amikacin. [11,12]

Klebsiella pneumonia is a gram-negative opportunistic bacterium responsible for community- and hospital-acquired infections. The mortality rate of neonatal sepsis caused by K. pneumonia ranges from 18% to 68%. [13] On local date reported in Yemen according to USTH in Sana'a period from 2006 to 2013. The most frequently as gram negative isolated species from the inpatients admitted to the departments of the USTH were E. coli and Acinetobacter species followed by Klebsiella species and P. aeruginosa.

However, the most frequently isolated Gram-negative bacteria, Acinetobacter species has the highest resistance rate to the most commonly used antibiotics, where only polymyxin B is effective against this species. P. aeruginosa shows an unchanging rate of resistance to antibiotics in the USTH despite being quite resistant to antibiotics on a global scale. [14]

Whereas anther study in Aden, Yemen Staphylococcus spp. followed by E. coli, Pseudomonas spp., and Klebsiella pneumonia, were the most widespread pathogenic bacteria in several isolates. Overall bacterial resistance was common for old antibiotics, such as the combination of sulfamethoxazole with trimethoprim, followed by amoxicillin and clavulanate. Additionally, cephalosporin had a relatively higher resistance rate than other antibiotics.

The study also showed moderate bacterial resistance toward gentamycin, azithromycin, cefoxitin, and ciprofloxacin However, a lower percentage of resistance was present for the combination of ampicillin with sulbactam, ertapenem, and levofloxacin. [18]

Pervious antibiogram that was done by clinical (antibiogram) Gram negative organisms E. Coli and Acinetobacter species were the most isolated gram negative (GN) bacteria, with 246(35.7%) and 148(21.4%) specimens, respectively. E. Coli was highly resistant to all antibiotics except Amikacin, Meropenem, Imipenem and Nitrofurantoin. E. coli had

susceptibilities 56% to gentamicin and 56% to Piperacillin /Tazobactam. Acinetobacter and klebsiella spp were highly resistant to all antibiotics.

Klebsiella pneumoniae had susceptibilities 59% to Amikacin, 66% to Imipenem and 71% to Meropenem. Pseudomonas aeruginosa had susceptibilities 64%to amikacin, 59%to gentamicin and 58% to Imipenem. Pseudomonas spp was highly resistant to all antibiotics except Imipenem. pseudomonas spp had susceptibilities 55% to Piperacillin /Tazobactam and 59% to Meropenem.

Gram positive organisms, There was 123(43.3%) coagulase negative staphylococci (CONS) isolates, 115(40.5%) S. aureus isolates, 23(8.1%) enterococcus spp isolates, 23(8.1%) streptococcus spp isolates CoNS had decreased susceptibility to Azithromycin(17%), Erythromycin (21%), Norfloxacin(25%) and Ampicillin /Sulbactam(45%) while maintaining susceptibility to Vancomycin, Linezolid, Imipenem, Amikacin. Enterococcus spp was highly resistant to all antibiotics except linezolid and Vancomycin. E. spp had susceptibility to Meropenem (60%).

S. Aureus had highly susceptibility to Imipenem and vancomycin. MRSA had highly resistant to all antibiotics except Vancomycin, linezolid and Co-Trimoxazole (see appendix B)

Important of antibiogram are useful in detecting potential infectious disease outbreaks the use of such aggregate data on local or regional resistance trends is fundamental to discern differences and changes in patterns for appropriate selection of antimicrobials for rational use and epidemiological surveillance [16,17]

(Objectives): Prevalence of most microorganism in neonate

Develop antibiogram for NICU.

Chapter Two

Literature Review

LITERATURE REVIEW

2.1 Infection in neonatal

Neonates are uniquely susceptible to infection. An immature immune system is coupled with exposure to the variety of maternal and environmental pathogens that can affect this population. [18]

Infants under the age of 3 months are rapidly building up their immunity by ramping up their production of immune cells and the creation of "memory" in their adaptive immunes systems through various exposures to their environment. During this period, the child is very vulnerable to serious bacterial infections such as those caused by group B streptococcus (GBS) and *E. coli*. [19]

Common infectious diseases affecting children include bronchiolitis, pneumonia, urinary tract infection, sinusitis, skin infection, gastroenteritis, and acute otitis media. [20] Whereas Common infectious diseases affecting in Neonatal sepsis and pneumonia Bloodstream infections (BSIs) are the most common HAIs in the NICU.

They can occur in isolation or in association with urinary tract infections (UTIs) and meningitis. Endocarditis, osteomyelitis, pyogenic arthritis, ventilator associated pneumonia, peritonitis, conjunctivitis, and skin abscesses are important, less common HAIs. [18]

Whereas viral infection, Neonates, like older children and adults, are subject to viral infections acquired by horizontal routes, such as those due to influenza, rotavirus, and enteroviruses.

They also are at risk for viruses through routes that are unique to the perinatal setting in which mother-to-child transmission can occur transplacentally, during birth, or from breast milk. The ability of cytomegalovirus (CMV), herpes simplex virus (HSV), human immunodeficiency virus (HIV), and human T-lymphotrophic virus type 1 (HTLV-1) to establish chronic infection in the mother with persistence of infectious virus in blood, mucosa, or milk accounts for the role vertical transmission plays in their epidemiology and potential clinical impact.

Whether viruses that produce acute, self-limited infections in the mother are transmitted to the fetus or newborn depends on the timing of maternal infection in relation to gestation

and parturition. The clinical settings in which fetal and neonatal viral infections must be considered include pregnancy, the newborn nursery, and the evaluation of an ill newborn.[18]

2.2 Antimicrobial Resistance (AMR)

Occurs when bacteria, viruses, fungi and parasites change over time and no longer respond to medicines making infections harder to treat and increasing the risk of disease spread, severe illness and death.

Mechanisms of Resistance .

1. Intrinsic resistance – Gram-negative bacteria have an outer membrane that makes them less permeable than gram-positive bacteria.
2. Adaptive resistance – Bacteria can adapt to their environment to survive. This occurs in the development of biofilms.
3. Acquired resistance – Most resistance that is of alarm is acquired resistance, with bacteria passing genetic material on plasmids.

a. Enzymes such as β-lactamases are passed on plasmids – Affected: β-Lactams.

b. Bacteria have reduced permeability or can use efflux pumps to push antibiotic out of the cell – Affected: Aminoglycosides, fluoroquinolones, macrolides, tetracycline.

c. Alteration in drug-binding target – Affected: β-Lactams, oxazolidinones, aminoglycosides, tetracycline, glycopeptides.

d. Alteration of the antibiotic – Affected: Fluoroquinolones, aminoglycosides, lincosamides

e. Bypass the effect of the antibiotic – Affected: β-Lactams, sulfamethoxazole/trimethoprim, glycopeptides [21].

Antimicrobial resistance classified into:

2.2.1 Resistance in Gram-Negative Infections

Mechanisms of Resistance.

The common thread in most of the organism threats is the presence of β-lactam resistance.

a) β-Lactams are the largest (and most prescribed) class of antimicrobials: penicillins, cephalosporins, monobactams, and carbapenems .

b) Three principles of acquired β-lactam resistance:

i) Decreased outer membrane penetration – Porin loss and increased drug efflux pumps .

ii) Alteration in binding target.

iii) Production of β-lactamases .

Extended-Spectrum β-Lactamases:

(1) Ability to hydrolyze penicillins, third-generation cephalosporins, and monobactams. Do not hydrolyze cephamycins and carbapenems.

(2) The three main families are TEM, SHV, and CTX-M. The most prevalent type is CTX-M, distributed worldwide and increasing in prevalence, found in E. coli and K. pneumoniae. Gene originally produced by bacteria Kluyvera .

(3) Originally associated with hospital-acquired outbreaks; now found in community-acquired infections.

(4) Associated with high mortality.

Carbapenems-Resistant Enterobacteriaceae

i) Rise in prevalence is related to the selective pressure from increasing carbapenems use.

ii) Carbapenems resistance in the United States is the combination of several mechanisms (e.g., ESBL plus poring loss), and these CRE do not constitute a carbapenems-producing Enterobacteriaceae (CPE). This distinction likely matters more epidemiologically than clinically because testing is not routine.

(a) True CPE likely has a very high carbapenem MIC, whereas CRE may have lower carbapenem MIC (though still resistant).

(b) CPE has been associated with higher mortality because it may represent a more virulent organism type.

iii) Organisms typically have acquired resistance to many classes by several mechanisms, and many isolates show as extremely drug resistant.

iv) Risk factors for poor outcomes include ICU admission, advanced age, requirements for vasopressors, mechanical ventilation, dialysis, non-urinary–sourced infections, and presence of colistin-resistant CRE. [22,23]

2.2.2resistance in Gram-Positive Infections

A. S. aureus Mechanisms of :

(1) Resistance has evolved for S. aureus depending on antibiotic exposure over time, starting with penicillin once exposed.

(a) Penicillin resistance developed 1 year after introduction of penicillin.

(b) Methicillin resistance developed 2 years after introduction of methicillin.

(2) MRSA

a. Penicillin resistance is caused by β-lactamase production (penicillinases). Methicillin is stable against hydrolysis by penicillinases.

b. Methicillin (and other β-lactam) resistance comes from alteration in the penicillin-binding protein (PBP) β-lactam–binding target to PBP2a, which is coded for by the mecA gene, carried on the staphylococcal cassette chromosome (SCC) SCCmec.

c. Theories regarding the origin of MRSA suggest that SCCmec was first transferred from coagulase-negative staphylococci.

(3) Vancomycin-resistant S. aureus(VRSA)

a. Occurrence is limited, with less than 20 incidents reported worldwide.

b. Transmission of VanA gene from Enterococcus to S. aureus. Despite VRE arising in the 1980s, transmission to S. aureus has not been commonplace

B. Enterococcus

Normal human flora in the gastrointestinal tract and a hardy, but nonvirulent microbe. Second to staphylococci in cause of hospital-acquired infection.

Capable of transmitting genes easily

Mechanisms of resistance

a. Ampicillin resistance

i. Most hospital-based E. faecium harbor an altered PBP5 that conveys ampicillin resistance.

ii. Enterococcus faecalis resistance is mediated by β-lactamase enzymes or alteration in PBP4.

b. Vancomycin resistance

More common in E. faecium than in E. faecalis

Alteration in the D-Ala-D-Ala binding site

(a) D-Ala-D-Lac is associated with high-level vancomycin resistance.

(b) D-Ala-D-Ser is associated with low-level vancomycin resistance [21]

Chapter Three

Methodology

Methodology

3.1 Patients and Study Site, stady design

The study was a retrospective study undertaken at USTH in Sana'a, Yemen. USTH is a private hospital 165-bed teaching hospital that trains medical students, nurses, and pharmacists and serves as a referral hospital for the country.

The collected data from bacterial isolates from Jan 2021 to March 2022 with susceptibility testing performed using diffuse disk by the microbiology laboratory from the Neonates intensive critical care (NICU) settings at USTH.

3.2 Lab Techniques

Specimens from blood, cerebrospinal fluid, sputum, urine, stool and swab were collected, processed, and analyzed in the microbiology laboratory according to the Kirby-Bauer method. The Kirby-Bauer test for antibiotic susceptibility (also called the *disc diffusion test*) is a standard that has been used for years.

3.3 Data Collection

The list of all isolates was collected from the system, then the sensitivity results for positive isolates were checked from the hospital records system. The data was entered into WHONET 5.6, a free Windows-based database software developed for the management of microbiology laboratory data. The data entered for each culture specimen included specimen number, sex, age category, department, specimen date, and organism. (See appendix B)

3.4 Data analysis

WHONET aggregated and analyzed the data. Getting Started-Setting up an analysis: %R, I, S and test measurements- Running the analysis and interpreting the Results-Transferring WHONET results to Excel and other software Susceptible Summary Isolate listing and summary (see Appendix C)

3.5 Antibiogram Development

Aggregated data from WHONET produced susceptibility percentages for every organism. The research team reviewed these auto-generated susceptibilities.

We initially excluded organisms not commonly associated with a disease or with fewer than 30 isolates, given the potential for diminished accuracy.[24]

We then reviewed the list and chose to include clinically important organisms despite having fewer than 30 isolates with the notation that these results should be interpreted with caution based on the low number of isolates.

The antibiotics included in the antibiogram were narrowed to those commonly available at USTH. We developed one antibiogram for pediatric doctors with specific percentage details for the most common antibiotics, and antibiotic sensitivity represented as resistant ("R"), intermediate ("I"), and sensitive ("S"). A common, but not universal, practice is to define susceptible as 80% to 100% susceptible, intermediate as 60% to 79.9% susceptible, and resistant as 0–59.9% susceptible.

We used these ranges in this antibiogram. In the instance of intrinsic resistance of an organism to an antibiotic, this was labeled as "R" rather than providing the percentage susceptible.

3.6 Ethics Statement

The study was approved by EIU and USTH. because this was a retrospective study of de-identified specimens, no consent form was required. (see appendix D)

Chapter Four

Result

Result

The number of screened patients were (257) with 324 isolated and out of them there were (133) patients with (148) positive isolation. The patients were screened from 2021/1/1 to 2022/3/31. The male represents 74% and female 26% (Table 1 gender numbers)

Table 1 gender of isolated

Sex	Frequency of isolated	(%)	Frequency of patients
M	109	74	97
F	39	26	36
Total	148	100	133

Table 2 showed that blood source represented the most isolation 133 (89.9%), while other sources (i.e. urine, swab, sputum, ….) were not very common source for isolation.

Table 2 The source of isolations with their Frequency and Percent .

Source	Frequency	Percent %
Blood	133	89.9%
Urine	4	2.7%
Swab	3	2%
Sputum	3	2%
Cerebrospinal fluid	2	1.4%
Other	2	1.4%
Stool	1	0.7%

4.1 Isolated and number of bacterial species that included in the antibiogram .

The total number of isolated after removal of rare bacteria isolated was 138 (G + ve (94 (68.1%%)), G –ve bacteria (44 (31.9%)). The results showed that G +ve bacteria was the most encounter isolated in USTH NICU, followed by G –ve bacteria.

Table 3 bacterial spcecies isolated and its number at NICU

Bacterial spcecies	No isolates	(%)	No patients	X^2 /*P*
Acinetobacter sp.	3	2.2	3	0.056/1
Burkholderia cepacia	9	6.5	8	
Enterobacter sp.	3	2.2	3	
Escherichia coli	6	4.3	6	
Klebsiella pneumoniae	5	3.6	5	
Klebsiella sp.	12	8.7	11	
Pseudomonas sp.	6	4.3	6	
Total of GN Isolates	44	32	42	

Table 4 bacterial spcecies isolated and its number at NICU

Bacterial spcecies	No isolates	(%)	No patients	X^2 / *P*
Staphylococcus aureus	6	4.3	6	1.734 /0.78445
Enterococcus sp.	6	4.3	6	
Staphylococcus hominis	4	2.9	4	
Staphylococcus coagulase negative	69	50	68	
Streptococcus sp.	9	6.5	7	
Total of GP Isolates	94	68	91	

4.1.1 Isolated of gram positive of bacterial species

4.1.1.1 staphylococcus coagulase negative: -

Staphylococcus was the most isolated G+ve 69 (50%) most antibiotic sensitive is linezolid and vancomycin as Table 5, The most

Table 5 Staphylococcus, coagulase negative sensitivety

Bacterial spcecies	Staphylococcus, coagulase negative		
AB	Total	No sensitive	Percent %
Amikacin	58	48	82.8
Azithromycin	54	10	18.5
Cefuroxime	56	23	41.1
Clindamycin	66	44	66.7
TMP/SMX	67	27	40.8
Erythromycin	69	10	14.5
Gentamicin	65	39	60
Linezolid	65	65	100
Penicillin	7	1	14.3
Tigecycline	4	4	100
Vancomycin	67	67	100

4.1.1.2 Streptococcus spp with was the 2nd isolated G +ve9 (6.5%) with sensitive to linezolid and vancomycin as is in (Table 6)

Table 6 Streptococcus sp sensitivity

Bacterial spcecies	Streptococcus sp		
AB	Total	No sensitive	Percent %
Azithromycin	6	5	86.3
Cefuroxime	9	6	66.7
Clindamycin	9	8	88.9
Linezolid	7	7	100
Penicillin G	9	7	77.8
Vancomycin	9	9	100

4.1.1.3 Staphylococcus aureus with 6(4.3%) isolate, the most sensitive was Linezolid, vancomycin and amikacin as is in (table 7)

Table 7 straphylococcus aureus sensitivty

Bacterial spcecies	Staphylococcus aureus		
AB	Total	No sensitive	Percent %
Amikacin	5	4	80
Azithromycin	6	1	16.7
Cefuroxime	6	2	33.3
Clindamycin	6	4	66.7
TMP/SMX	6	2	33.3
Erythromycin	6	1	16.7
Gentamicin	6	4	66.7
Linezolid	6	6	100
Vancomycin	6	6	100

4.1.1.4 Enterococcus sp is with 6 (4.3%) isolate, the most sensitive was linezolid and vancomycin as is in (table8)

Table 8 Enterococcus sp. sensitive

Bacterial spcecies	Enterococcus sp.		
AB	Total	No sensitive	Percent %
TMP/SMX	6	0	0
Erythromycin	4	2	50
Linezolid	6	6	100
Penicillin G	6	2	33.3
Vancomycin	6	6	100

4.1.1.5 Staphylococcus hominis which is the less bacterial in gram +ve with 4(2.9%) with the most sensitive is linezolid, vancomycin and tigecycline as in (table9)

Table 9 Staphylococcus hominis sinsitive

Bacterial spcecies	Staphylococcus Hominis		
AB	Total	No sensitive	Percent %
Clindamycin	4	1	25
TMP/SMX	4	3	75
Erythromycin	4	0	0
Gentamicin	4	2	50
Linezolid	4	4	100
Tigecycline	4	4	100
Vancomycin	4	4	100

4.1.2. Overall suspitibility of Gram-Positive Organisms

The above showed that There were (69 (50%)), Staphylococcus species, coagulase negative (CoNS) isolates, 9(6.5%) Streptococcus spp, 6(4.3%) S. aureus isolates, 6(4.3%) Enterococcus isolates, and 4(2.9%) Staphylococcus hominis. S had decreased susceptibility to Erythromycin 69(14.5%), while maintaining susceptibility to vancomycin, linezolid, while other antibiotics such as trimethoprim and cephalosporin were resistance to most gram + bacteria .

Table 10 antibiogram of gram positive

Organism	Orga nism	Amikacin n(%)	Azithromycin %S	Cefepime %S	Cefixime %S	Cefoperazone %S	Cefotaxime %S	Ceftazidime %S	Cefuroxime %S	Clindamycin %S	Colistin %S	Trimethoprim/Sulfameth oxazole %S	Erythromycin %S	Gentamicin %S	Imipenem %S	Linezolid 10 %S	Penicillin G 1 unit %S	Piperacillin/Tazobacta m 100/10 %S	Tigecycline %S	Vancomycin 5 %S	Polymyxin B %S	Meropenem %S	Ceftriaxone %S
Staphylococcus aureus	6	S	R						R	I		R	R	I		S				S			
Staphylococcus, coagulase negative	69	S	R	R					R	I		R	R	I		S	R		S	S		S	
Staphylococcus hominis	4									R		I	R	R		S			S	S			
Streptococcus sp.	9		S						I	S		I	S			S	I			S			
Enterococcus sp.	6											R	R			S	r			S			

Colors green = sensitive (80-100%) yellow = intermittent (60-79.9%) red = resistance (0-59.9)

4.1.3 Gram negative bacterial species

4.1.3.1 The number of isolated bacteria of Acinetobacter spp was 3(2.2%) and the most sensitive antibiotic for it were Colistin Imipenem… as in (table 11)

Table 10 Acinetobacter sensitive

Bacterial species	Acinetobacter sp		
AB	Total	No sensitive	Percent %
Amikacin	3	1	33.3
Cefepime	2	0	0
Cefotaxime	3	0	0
Ceftazidime	3	0	0
Colistin	2	2	100
TMP/SMX	2	1	50
Gentamicin	2	0	0
Imipenem	3	3	100
Piperacillin/Tazobactam	3	3	100
Polymyxin	1	1	100
Meropenem	3	3	100
` Ceftriaxone	2	0	0

4.1.3.2 the number of isolated bacteria of Escherichia coli was 6(4.3%), the most sensitive Amikacin, Gentamicin…etc. as in (table 12)

Table 11 Escherichia coli sensitive

Bacterial species	Escherichia coli		
AB	Total	No sensitive	Percent %
Amikacin	5	5	100
Cefepime	6	1	16.6
Cefixime	2	0	0
Cefoperazon	3	0	0
Cefotaxime	3	1	33.3
Ceftazidime	6	1	16.6

Cefuroxime	4	0	0
TMP/SMX	5	1	20
Gentamicin	6	6	100
Imipenem	5	5	100
Piperacillin/Tazobactam	4	2	50
Meropenem	6	6	100
Ceftriaxone	6	1	16.7

4.1.3.3 The number of isolated bacteria of Enterobacter spp is bacteria from was 3(2.2%), The most antibiotic sensitive is in (table 13)

Table 12 Enterobacter sp sensitive

Bacterial species	Enterobacter spp		
AB	Total	No sensitive	Percent %
Amikacin	2	1	50
Cefepime	2	0	0
Cefixime	1	0	0
Cefotaxime	2	1	50
Ceftazidime	2	0	0
Cefuroxime	2	0	0
TMP/SMX	3	2	66.7
Gentamicin	2	1	50
Imipenem	3	2	66.7
Penicillin G	1	1	100
Piperacillin/Tazobactam	2	1	50
Meropenem	2	1	50
Ceftriaxone	2	0	0

4.1.3.4 Klebsiella spp is the most gram negative bacterial found in this study with number of isolate 12(8.7%), the most sensitive antibiotic colistin and polymyxin as in (table 14)

Table 14 Klebsiella sp sensitive

Bacterial species	Klebsiella sp		
AB	Total	No Sensitive	Percent %
Amikacin	12	7	58.3
Cefepime	12	1	8.3
Cefixime	3	0	0
Cefoperazon	6	0	0
Cefotaxime	4	1	25
Ceftazidime	12	0	0
Cefuroxime	6	0	0
Colistin	1	1	100
TMP/SMX	11	7	63.6
Gentamicin	11	4	36.4
` Imipenem	7	6	85.7
Piperacillin/Tazobactam	7	4	57.1
Polymyxin B	1	1	100
Meropenem	12	7	58.3
Ceftriaxone	11	0	0

4.1.3.5 the number of isolated bacteria of Klebsiella pneumonia is was 5(3.6%) isolate, the most sensitive antibiotic is in (table 15)

Table 15 Klebsiella pneumonia sensitive

Bacterial species	Klebsiella pneumoniae		
AB	Total	No sensitive	Percent %
Amikacin	5	5	100
Cefepime	5	1	20
Ceftazidime	5	1	20
TMP/SMX	5	4	80
Meropenem	5	4	80
Ceftriaxone	5	1	20

4.1.3.6 The number of isolated bacteria of Pseudomonas spp was 6(4.3%) isolates, the most sensitive antibiotic is in (table 16)

Table 13 Pseudomonas sp sensitive

Bacterial species	Pseudomonas sp		
AB	Total	No sensitive	Percent %
Amikacin	6	3	50
Cefepime	5	1	20
Cefoperazon	1	0	0
Ceftazidime	6	1	16.7
Cefuroxime	1	0	0
Gentamicin	6	3	50
Meropenem	6	6	100
Ceftriaxone	5	2	40

4.1.3.7 Burkholderia cepacia is 2nd gram negative Bactria in this study with (9(6.5%)) isolated and the most sensitive antibiotic is in (table 17)

Table 14 Burkholderia cepacia sensitive

Bacterial species	Burkholderia cepacia		
AB	Total	No sensitive	Percent %
Ceftazidime	9	4	44.4
TMP/SMX	9	6	66.7
Meropenem	8	8	100

4.1.4 The above showed that Gram-Negative Bacteria

Klebsiella spp and Burkholderia cepacia were the most isolated Gram negative (GN) bacteria, with 12(8.7%) and 9(6.5%) specimens, they were highly resistant to all antibiotics except Imipenem, Polymyxin B and Colistin for Klebsiella spp and Meropenem, Trimethoprim/Sulfamethoxazole for Burkholderia cepacia. Klebsiella spp had susceptibilities of Piperacillin/Tazobactam7(57.1%), Cefotaxime 4(25%) and Gentamicin 11(36.4%). The high resistance to 3rd. generation cephalosporin indicated high rates of beta-lactamase production including ESBLs

Table 15 Antibiogram for gram negitive

Organism	Org	Amikacin n(%)	Azithromycin %S	Cefepime %S	Cefixime %S	Cefoperazone %S	Cefotaxime %S	Ceftazidime %S	Cefuroxime %S	Clindamycin %S	Colistin %S	Trimethoprim/Sulfameth oxazole	Erythromycin %S	Gentamicin %S	Imipenem %S	Linezolid 10 %S	Penicillin G 1 unit %S	Piperacillin/Tazobactam 100/10	Tigecycline %S	Vancomycin 5 %S	Polymyxin B %S	Meropenem %S	Ceftriaxone %S
Acinetobacter sp.	3	R		R			R	R			S	R		r	S			S			S	S	R
Escherichia coli	6	S		R	R	R	R	R	R			R		S	S			R				S	R
Enterobacter sp.	3	R		R	R		R	R	R			I		R	I		S	R				R	R
Klebsiella sp.	12	R		R	R	R	R	R	R		S	I		R	S			R			S	R	R
Klebsiella pneumoniae	5	S		R				R				R		S								S	R
Burkholderia cepacia	9							R				I										S	
Pseudomonas sp.	6	R		R		R		R	R					R	S			S				S	R

Colors green = sensitive (80-100%), yellow = intermittent (60-79.9%), red = resistance (0-59.

Chapter Five

Discussion

Discussion

In the study, were analyzed neonate bacterial isolates from USTH at NICU and most important findings were as following: (1) G +ve were the most isolated bacteria among neonate patients; (2) the most sensitive antibiotics for gram +ve were linezolid and vancomycin; (3) The most isolated g –ve bacteria were resistance to most cephalosporin generations and sensitive mostly for carbapenem antibiotics.

In this study the gram positive was the most isolated bacteria in USTH hospital institution the most common isolate Staphylococcus coagulase negative 69 (50%). however, in other studies that were conducted at NICU form Zambia, (25) and in Yemen; was in Sana'a (26).

They found the gram negative was the most prominent bacteria. The high percentage of gram positive in our institution may be due to contaminated but we cannot confirm that in our study as retrospective study design. Other factors may be due to different locations, populations, and clinical situation. For the gram-positive bacteria, the most sensitive antibiotic vancomycin and linezolid and resistance to cephalosporin, Erythromycin and TMP/SMX. Similar to our finding, studies that was conducted in Yemen, and Zambia found that TMP/SMX and erythromycin were resistance as well. The most sensitive antibiotics in our study and mention studies were vancomycin and linezolid. Staphylococcus resistance to TMP/SMX and erythromycin indicates the isolated Staphylococcus was MRSA. this is indicted for staph auras the MRSA the most commonly encounter bacteria and this is may be duo to result from the overuse of antibiotics.

Our findings of high resistance to Erythromycin and TMP/SMX are consistent with other studies. These findings are concerning because TMP/SMX is commonly used for empiric treatment, particularly respiratory and gastrointestinal infections.

The gram negative isolates showed high level of sensitivity to carbapenem and high resistance to cephalosporin which was consistent with other studies that were conducted in Zambia, and this is maybe indicate of ESBL for these isolated.

5.2 Limitation

This study had some limitations. First, this retrospective study is based on the data collected form laboratory records which lack information about the neonates' hospitalization date, clinical information, and treatment outcome. Therefore, we were not able to classify infections as community acquired or hospital acquired infection. Similarly, we could not determine whether the antibiotic resistance was primary or secondary resistance. Moreover, data on the clinical information and treatment outcome of the neonates were not included in this study. Second, this study was conducted only at a single hospital; therefore, the antibiotic resistance patterns observed in our study might not generalize the situation in the country.

Chapter Six

Conclusions and Recommendation

6.1 Conclusions

The resistance for antimicrobial gram positive and gram negative still high in neonate as only vancomycin and linezolid was most sensitive for the gram positive and carbapenem and Colistin only the sensitive antibiotic for gram negative

6.2 Recommendation

Antibiogram is necessary for neonate as national level not local level prospective Should be restriction use of unnecessary antibiotic and awareness healthcare and student about resistance because there is high rate of resistance among neonate

Focus the infection control to limited the separate the most resistance bacteria

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APPENDICES

Appendix A Pervious Work

University of Science and Technology Hospital

Antibiotics Susceptibility %

(from 1 / 5 /2015 to 30 / 4 /2016)

Types / Microorganisms / Antimicrobial	NO. Isolates [1,2]	Amikacin	Gentamicin	Amoxicillin/ Clavulinic Acid	Ampicillin	Ampicillin / Subactam	Penicillin	Piperacilin / Tazobactam	Methicillin	Azithromycin	Erythromycin	Clindamycin	Imipenem	Meropenem	Cefepime	Ceftxime	Cefoperazone / Subactam	Cefoxitin	Cefpirome	Cefpodoxime	Ceftazidime	Ceftriaxone	Cefuroxime	Ciprofloxacin	Levofloxacin	Moxifloxacin	Colistin	Polymyxin B	Doxycycline	Tetracycline	Co-Trimoxizole	Nitrofurantoin	Vancomycin	Tigecycline	Linezolid	Rifampicin
Gram Positive																																			
Staphylococcus aureus	66		78	61		83		78.5	50		66	87		75									76		76	85			80.5	55					76
Coagulase negative Staphylococci	47			51			66.6	60				74.3	86.6				65							53	61				66.6						
Streptococcus spp.	14		85									85																90		76				
MRSA-Methicillin Resistant Staphylococcus aureus	10													50																	50			
Enterococcus faecalis	2																									50								
Gram Negative																																			
Escherichia coli	109							63.9																				83			73.6			
Klebsiella pneumoniae	67	66	60										59	45			53.6									59			60					
Acinetobacter baumannii	64																																		
Pseudomonas aeruginosa	35	68	76										64	58	54		86							56		68								
ESBL-E.coli	22							59																		75					80.9			
Acinetobacter	21																										87								
Pseudomonas Spp	10	75	80	50	50											50	66.6							87	85	71			66.6		70				

Valid Until 4 / 2017

[1] Number of isolates may vary with each antimicrobial

[2] For isolates < 30 result must be interpreted with caution

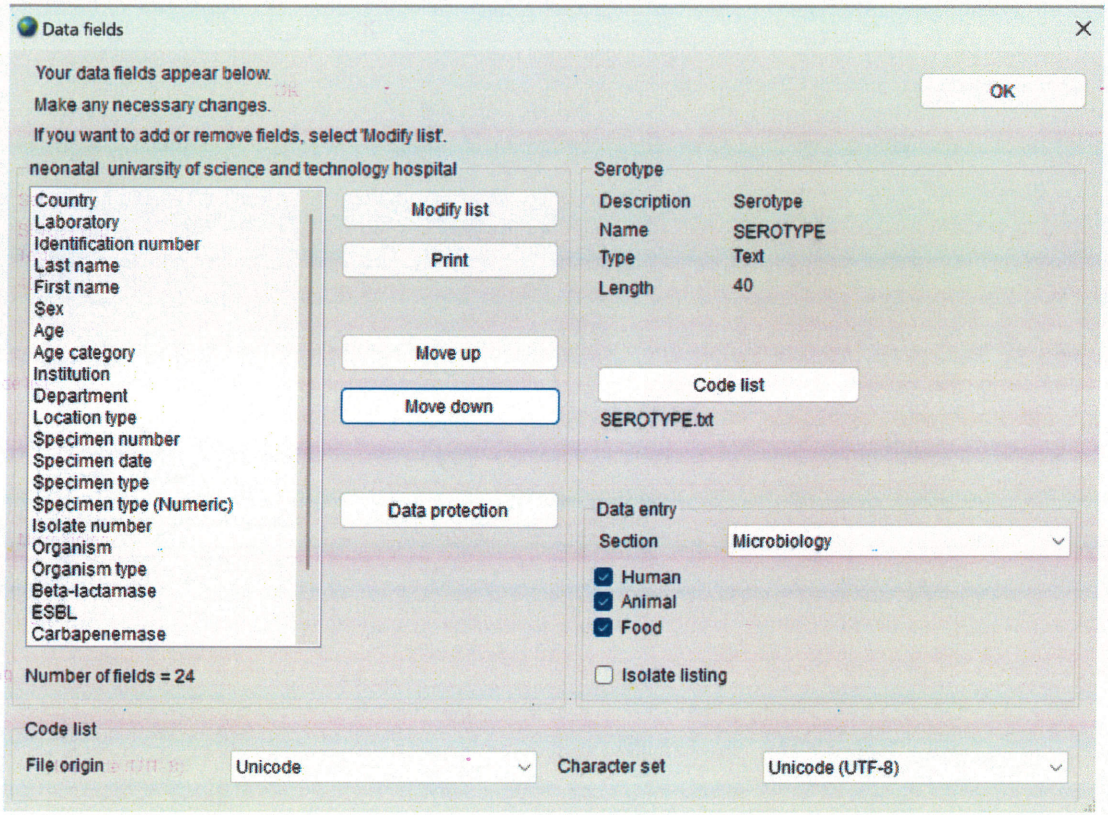
Most strains susceptible > 90%

Many strains susceptible 50 - 90%

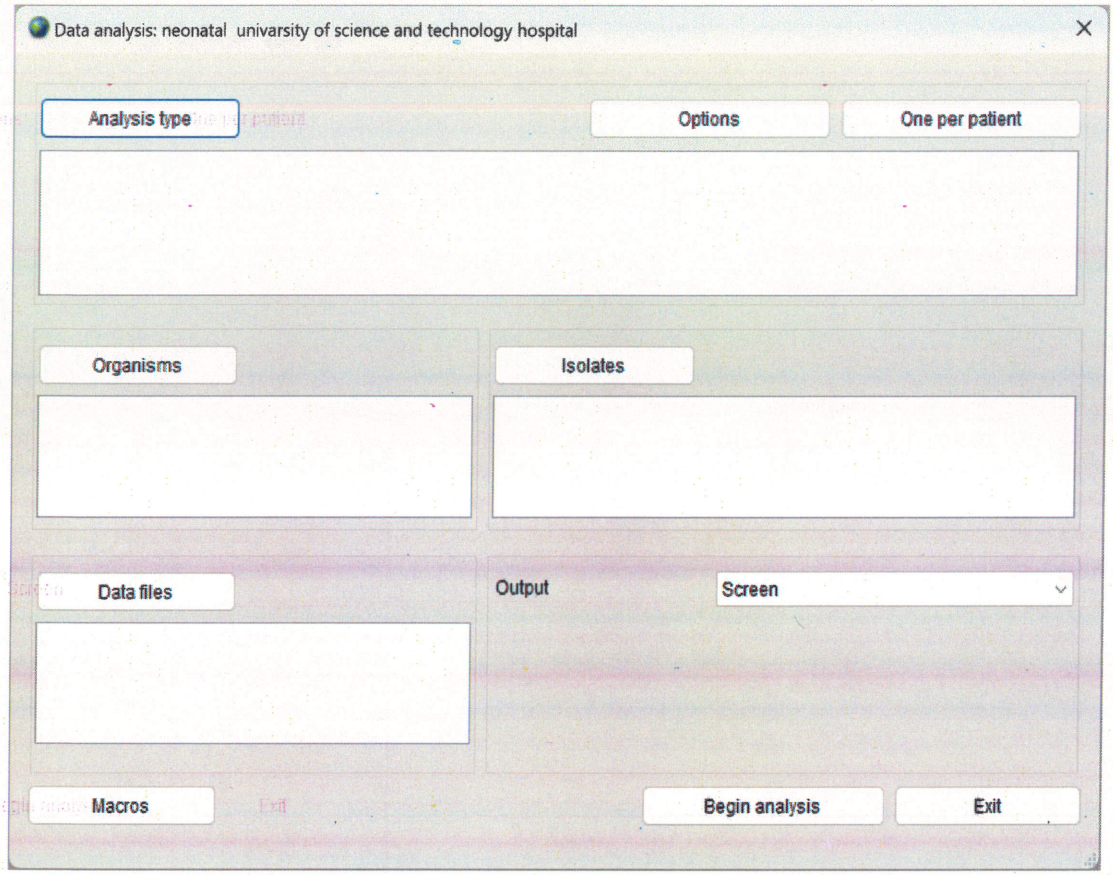
few strains susceptible < 50%

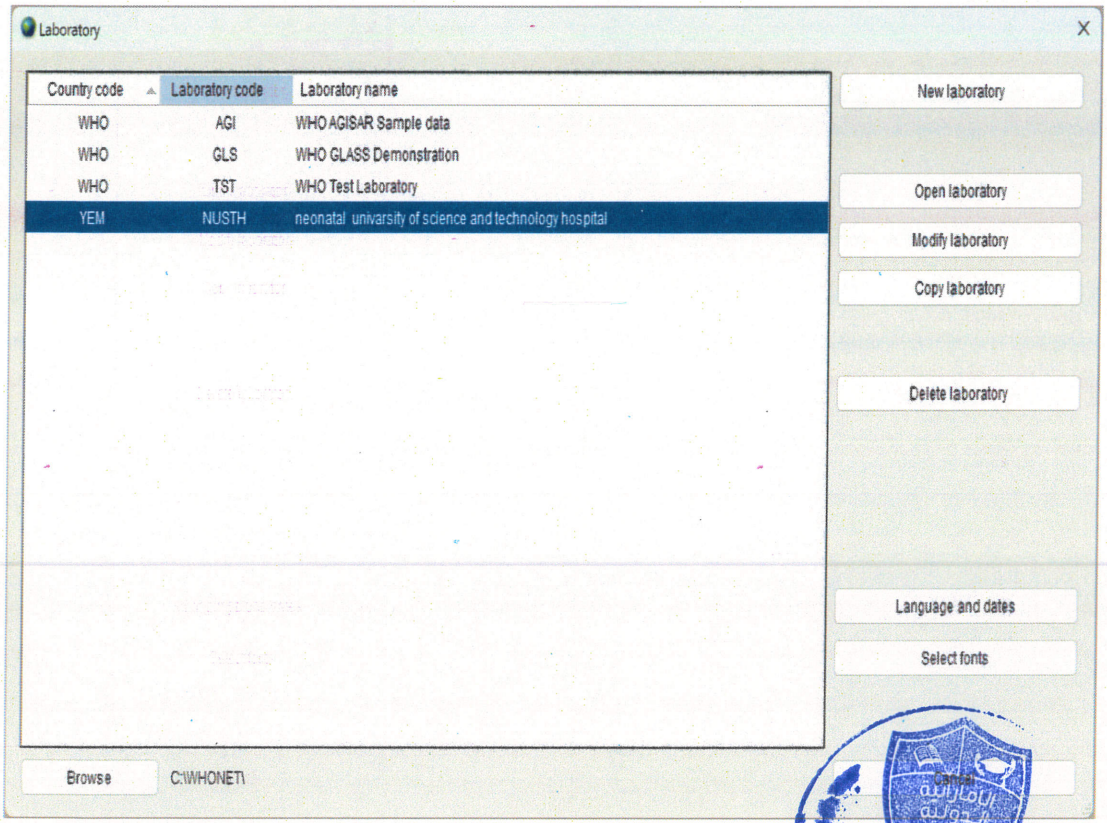
Not determined

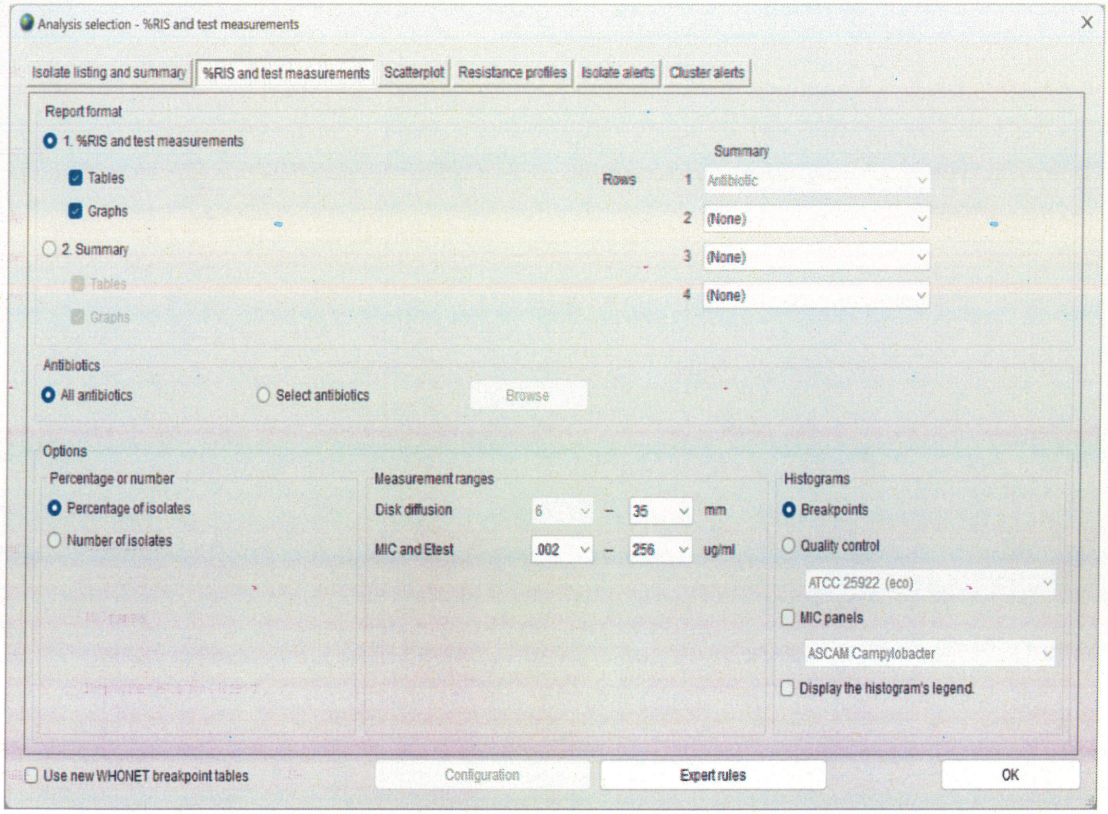
Appendix B Data collected

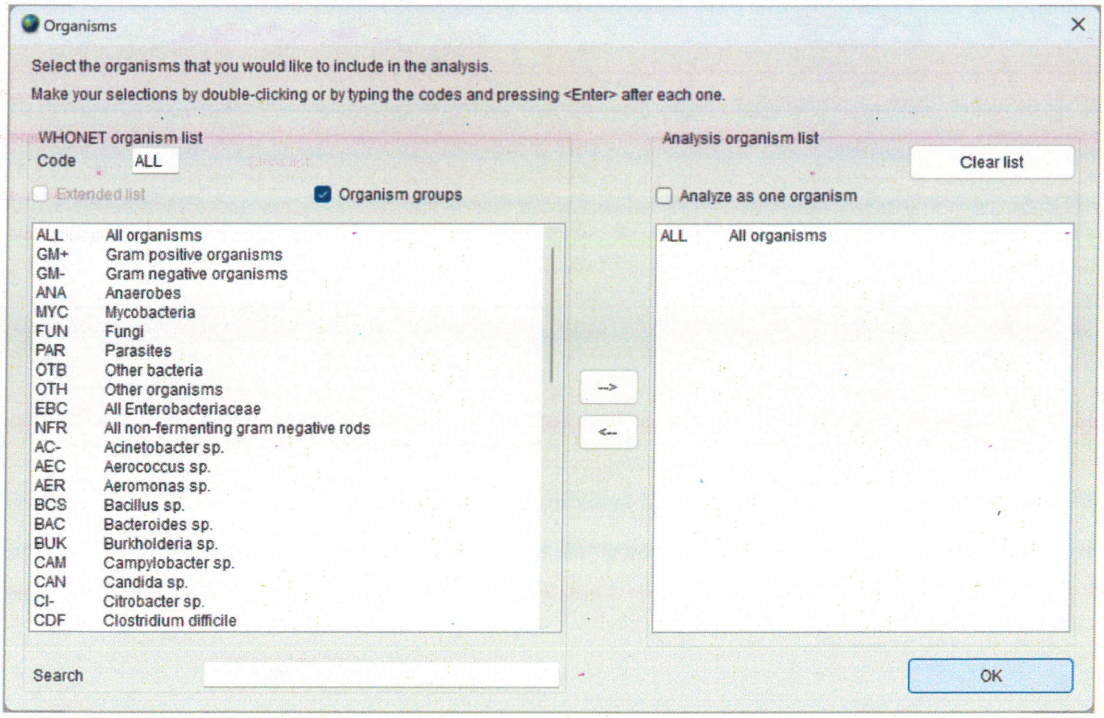


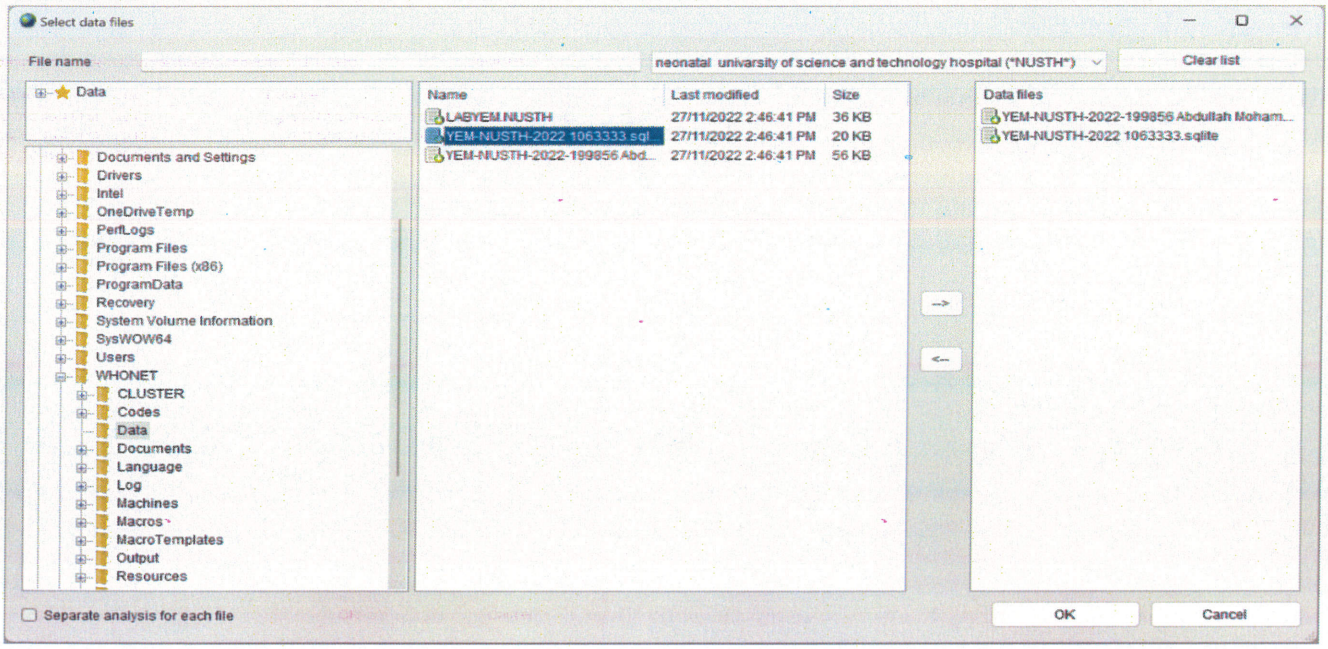
Appendix C Data analysis

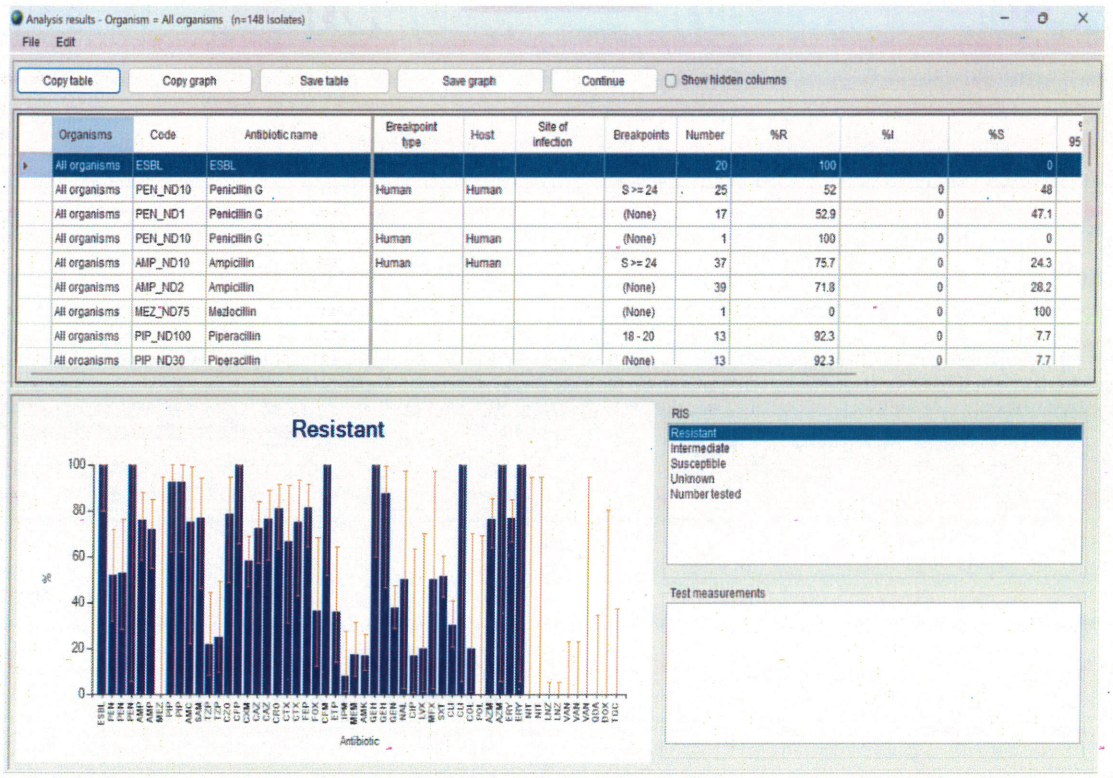






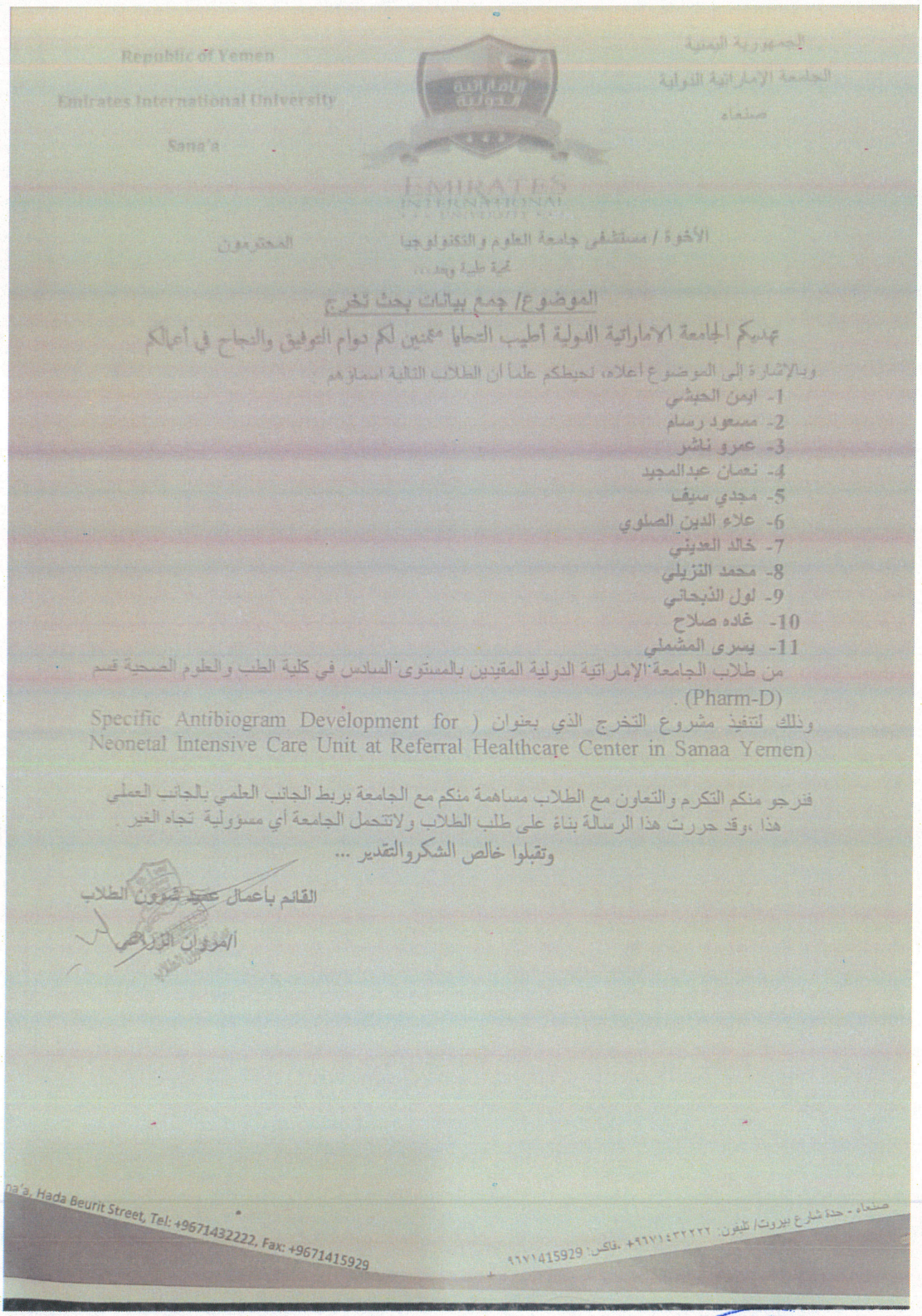






Organisms	Code	Antibiotic name	Breakpoint type	Host	Site of infection	Breakpoints	Number	%R	%I	%S	95
All organisms	ESBL	ESBL					20	100		0	
All organisms	PEN_ND10	Penicillin G	Human	Human		S >= 24	25	52	0	48	
All organisms	PEN_ND1	Penicillin G				(None)	17	52.9	0	47.1	
All organisms	PEN_ND10	Penicillin G	Human	Human		(None)	1	100	0	0	
All organisms	AMP_ND10	Ampicillin	Human	Human		S >= 24	37	75.7	0	24.3	
All organisms	AMP_ND2	Ampicillin				(None)	39	71.8	0	28.2	
All organisms	MEZ_ND75	Mezlocillin				(None)	1	0	0	100	
All organisms	PIP_ND100	Piperacillin				18 - 20	13	92.3	0	7.7	
All organisms	PIP_ND30	Piperacillin				(None)	13	92.3	0	7.7	

Appendix D Premission letter of EIU



Republic of Yemen
Emirates International University
Sana'a

الجمهورية اليمنية
الجامعة الإماراتية الدولية
صنعاء

EMIRATES INTERNATIONAL UNIVERSITY

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الموضوع/ جمع بيانات بحث تخرج

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فنرجو منكم التكرم والتعاون مع الطلاب مساهمة منكم مع الجامعة بربط الجانب العلمي بالجانب العملي هذا ،وقد حررت هذا الرسالة بناءً على طلب الطلاب ولاتتحمل الجامعة أي مسؤولية تجاه الغير .

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القائم بأعمال عميد شؤون الطلاب

المروان الزراعي

Sana'a, Hada Beurit Street, Tel: +9671432222, Fax: +9671415929

صنعاء - حدة شارع بيروت/ تليفون: ٤٣٢٢٢٢ ٩٦٧١+ ،فاكس: ٩٦٧١٤١٥٩٢٩

EMIRATES INTERNATIONAL UNIVERSITY

الخلاصة

خلفية

مناعة حديثي الولادة منخفضة للغاية ويمكن أن تؤدي العدوى فيما بينها إلى عواقب تهدد الحياة. تعتبر مقاومة المضادات الحيوية واحدة من أكثر المجالات تحديا في علاج الأمراض المعدية في جميع أنحاء العالم ويمكن أن تؤدي إلى فشل العلاج بين حديثي الولادة.

الهدف

كان الهدف العام من هذه الدراسة هو تطوير مضاد حيوي خاص بحديثي الولادة في مستشفى جامعة العلوم والتكنولوجيا، صنعاء، اليمن.

المنهجية

هذه دراسة بأثر رجعي، تم جمع البيانات من قاعدة بيانات مستشفى جامعة العلوم والتكنولوجيا وتم استخدام برنامج WOHNETلتحليل وتطوير مخطط المضادات الحيوية في مستشفى جامعة العلوم والتكنولوجيا

نتيجة

كان البكتيريا موجبة الجرام هي الأكثر شيوعا من البكتيريا التي تواجهها بين حديثي الولادة، تليها البكتيريا سالبة الجرام، من البكتيريا موجبة الجرام كانت المكورات العنقودية السلبية والمكورات العقدية هي الأكثر شيوعا، وكانت الكليبسيلا والبروكولديريا هي الأكثر شيوعا في البكتيريا موجبة الجرام، وكان المضاد الحيوي الأكثر حساسيه بالنسبة للبكتيريا موجبه الجرام هما الفانكومايسين والينزولايد، في حين أن الكربابينيم والكوليستين في البكتيريا سالبة الجرام

الاستنتاج

المقاومة لعامل مضاد الميكروبات كانت عالية في حديثي الولادة وتشمل البكتيريا موجبة وسالبة الجرام.