MICROBIOLOGICAL STUDIES ON MULTI-DRUG RESISTANT BACTERIA THAT CAUSE NEONATAL SEPTICEMIA IN EGYPT

A thesis

Submitted in Partial Fulfillment of the Requirements for
The Master degree in Pharmaceutical Sciences
(Microbiology & Immunology)

Presented by

Manal Mahmoud Amer Ismael Fayed

B. Sci. Of Pharmaceutical Sciences for the partial fulfillment of the Master degree in microbiology & Immunology.

Under the supervision of:

Prof. Dr. Fatheya El Dessooky Ahmed

Prof. of Microbiology& Immunology Department Faculty of Pharmacy, Cairo University

Prof. Dr. AmalEmad El Din Mohammed

Prof. of Microbiology& Immunology Department Faculty of Pharmacy, Cairo University

Prof. Dr. Hayam Mahmoud Hussien

Prof. of microbiology in National Organization of Drug Control and Research (NODCAR).

DepartmentofMicrobiology& Immunology

Faculty of Pharmacy

Cairo University

2018

Approval Sheet

MICROBIOLOGICAL STUDIES ON MULTI-DRUG RESISTANT BACTERIA THAT CAUSE NEONATAL SEPTICEMIA IN EGYPT

Thesis submitted by

Manal Mahmoud Amer Ismael Fayed.

B. Sci. of Pharmaceutical Sciences for the partial fulfillment of the Master degree in microbiology & Immunology.

Approved by:

Prof. Dr. Fatheya El Dessooky Ahmed……...

Prof. Dr. AmalEmad El Din Mohamed…….

Prof. Dr. Hayam Mahmoud Hussien……..

Prof. Dr.Mohamed Abdel-halem…….

Prof. Dr. HanaaAbdelfatahElsaid Mansour……...……...

Date: / / 2018

Acknowledgment

First and foremost, thanks to ALLAH, the most beneficent, the most merciful.

I would like to express my deepest thanks and sincere gratitude to my **Prof. Fatheya El Dessooky,** Professor of Microbiology and Immunology, Faculty of Pharmacy, Cairo University for her vision, kind supervision, wise guidance and valuable advice. I was honored to work with such a great supportive person.

I would like to thank the rest of my thesis committee: **Prof. Amal Emad El Din (**Professor of microbiology and immunology, Faculty of Pharmacy, Cairo University) and **Prof. Hayam Mahmoud Hussein** (Professor of Microbiology, National Organization of Drug Control and Research, NODCAR) for their insightful comments and encouragement, but also for the hard question which incented me to widen my research from various perspectives.

I would like to express my sincere gratitude to my advisor **Assoc. Prof. Dr. Wael Mohamed Abu El-Wafa Noor El-deen**for the continuous support of my M. Sci. study and related research, for his patience, motivation, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis and I could not have imagined having a better advisor and mentor for my M. Sci. study.

I would like to thank **Sigma** to help me the identification of most resistant isolates.

I am also grateful to the members of microbiology labs (A and C) in National Organization for Drug Control and Research (NODCAR) for participation in the survey who supported my work in this way and helped me get results of better quality

Last but not the least, I would like to thank my family: my parents and to my brothers and sister for supporting me spiritually throughout writing this thesis and my life in general.

Microbiological Studies on Multi-Drug Resistant Bacteria that Cause Neonatal Septicemia in Egypt

Manal Mahmoud Amer Ismael Fayed
National Organization for Drug Control and Research

ABSTRACT

Neonatal sepsis is a leading cause of neonatal mortality in developing countries. Identification of the etiological agents of neonatal sepsis is essential for effective treatment. Out of 106 microbial isolates recovered blood cultures of neonatal sepsis patients, seventy (66.1 %) isolates of them were Gram positive bacteria, 31 (29.2 %) isolates were belonging to Gram negative bacteria and 5 (4.7%) isolates were belonging to *Candida* sp.Coagulase negative staphylococci (CONS) was the most common causative late onset neonatal septicemia (LOS), which reached to 43(40.6%) of total isolates, followed by *Micrococcus*, *Enterobacter*, coagulase positive staphylococci (COPS),*Candida*, *Shigella*, *E. coli*, *Bacillus*, *Citrobacter*and*Klebsiella* isolates, which reached to 13(12.2%), 11(10.4%), 10(9.4%), 7(6.6%), 5(4.7%), 5(4.7%), 4(3.7%),4(3.7%) and 4(3.7%), respectively. CONS isolates were highly resistant to various tested antibiotics compared to COPS. In addition, 50% of *Staphylococcus* isolates were resistant to aminoglycosides, IPM, glycopeptides and linezolid antibiotics, while 50% ofenterobacterial isolates were resistant to glycopeptides, aminoglycosides, monobactam and tetracycline. The most resistant strains in the present study were *Enterobacter cloacae*NBS-40neonatal blood samplesNBS-40, *S. aureus subsp. aureus*NBS-35 and *S. epidermidis*NBS-98. The interaction of double antibiotics combinations against these strains were investigated by checkerboard method and the results showed that an antagonism was the common form of combination interaction followed by synergism and indifferent interactions. *S. epidermidis*-NBS-098 was the most sensitive strain to the tested combinations, followed by *E. cloacae*-NBS-040 and *S. aureus*-NBS-035, which reach their synergistic responses to 50, 41.4 and 33.33% of total tested combinations, respectively. *B*-lactam and aminoglycoside antibiotics were the most common constituent of antibiotic combinations that showed synergistic interaction against various tested strains.

LIST OF ABBREVIATIONS

Abbreviation	Title
A/A	**Acid slant/ Acid butt……..…….....**
A/ALK	**Acid butt /Alkaline slant…….**
A/G	**Acid and gas…….**
AK	**Amikacin……..**
AMC	**Amoxicillin/clavulanic acid…….**
AMP	**Ampicillin…….**
BAM	**Blood agar medium…….**
BLAST	**Basic local alignment search tool……...**
B-P	**Barid-Parker agar medium……**
CA	**Ceftazidime……..**
CAT	**Catalase test…….**
CIP	**Ciprofloxacin……...**
CIT	**Citrate utilization test…….**
CN	**Gentamicin……...**
CLR	**Clarithromycin……**
CO	**Coagulase test……..**
CONS	**Coagulase negative staphylococci……..**
COPS	**Coagulase positive staphylococci……...**
CUA	**Christensen's urea agar medium……...**
CPM	**Cefepime……...**
CTX	**Cefotaxime……...**
DA	**Clindamycin……..**
DO	**Doxycycline……..**
EMB	**Eosin methylene blue medium……**

F	**Female……..**
FIC	**Fractional inhibitory concentration……...**
CGC	**Chiny green colonies……...**
I	**Intermediate…….**
IND	**Indole test…….**
IPM	**Imipenem……..**
LF	**Lactose fermenter……**
LN	**Lactose non –fermenter……..**
LONS	**Late onset neonatal septicemia……...**
LZD	**Linozolid……...**
M	**Male……..**
MAM	**MacConkey's agar medium……**
MHA	**Muller-Hinton agar medium……..**
MHB	**Muller-Hinton broth medium……**
MIC	**Minimum inhibitory concentration……...**
MOT	**Motility test……..**
MR	**Methyl red test…….**
MSA	**Mannitol salt agar medium……**
MR-VP	**Methyl Red-Vogues-Proskeur broth…….**
NA	**Naldixic acid…….**
NBS	**Neonatal blood sample……**
NCBI	**National center for biotechnology information institute….**
ND	**Not determined……**
NOR	**Norfloxacin……...**
NR	**Nitrate reduction test……..**
NTB	**Nitrate broth medium…….**

NUA	**Nutrient agar medium……**
NUB	**Nutrient broth medium……...**
OFX	**Ofloxacin……...**
OX	**Oxacillin……**
OXI	**Oxidase test……..**
P	**Penicillin……...**
R	**Resistant……**
S	**Sensitive……**
SAM	**Ampicillin/sulbactam……..**
SCA	**Simmons citrate agar medium……**
SDA	**Sabouraud agar medium…….**
16SrRNA	**16 subunit ribosomal ribonucleic acid……...**
SSM	**Semisolid medium……**
ST	**Streptomycin……**
T	**Tetracycline……..**
TOB	**Tobramycin……..**
TPZ	**Piperacillin/tozobactam……...**
TSI	**Triple sugars iron agar medium…….**
URE	**Urease test…….**
VA	**Vancomycin……..**

LIST OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1-INTRODUCTION

Sepsis is a significant cause of morbidity and mortality in thenewborn, particularly in preterm, low birth weight infants, despiteadvances in neonatal care, infections remain common and sometimeslife-threatening in neonates admitted to the neonatal intensive care unit (NICU) **(Stoll *et al*., 2010).**

Neonatal sepsis is a common life threatening disease with an incidence of 3.5 to 8 cases per 1,000 live births and a mortality rate of 16 to 30%, and accounts for 1.6 million deaths annually in developing countries **(El-Mazary*et al*.,2010)**. Three-fourths of the 4 million neonatal deaths occur during the first 7 days after birth **(Haque, 2004).**

In 2010 worldwide, 7.6 million children less than 5 years old died, predominantly because of infectious causes including sepsis; neonatal deaths (in the first 28 days of life), accounted for 40% of the total lives lost **(Liu*et al*., 2012)**.

Neonatal sepsis refers to systemic infection of the newborn. It is characterized by a constellation of a nonspecific symptomatology in association with bacteremia. Prompt recognition, appropriate antimicrobial therapy and judicious supportive care are the key determinants of positive outcome in this serious pediatric emergency. In developing countries, sepsis including meningitis, respiratory infections, diarrhea, and neonatal tetanus is the commonest cause of mortality responsible for 30-50 % of 5 million total neonatal deaths each year. It is estimated that almost 20 % of all neonates develop infection. **(Paul and Singh, 2000 and Remington *et al*., 2006).**

Neonatal sepsis can be classified into two subtypes depending upon onset of symptoms. It may be categorized as Early onset sepsis(EOS) or Late onset sepsis (LOS). In caseof newborns with early-onset sepsis, 85% present within 24 hours, 5% present at 24-48 h, and a smaller percentage present within 48-72 hours. Onset is most rapid in premature neonates **(Klinger*et al*., 2009).**

LOS (Late onset neonatal septicemia) usually presents after 72 h of age. The source of infection in LOS is either nosocomial (hospital-acquired) or community - acquired and neonates usually present with septicemia, pneumonia or meningitis. **(Wolach, 1997 and Baltimore, 1998).** Various factors that predispose to an increased risk of nosocomial sepsis include low birth weight, prematurity, admission in intensive care unit, mechanical ventilation, invasive procedures, administration of parenteral fluids, and use of stock solutions. Factors that might increase the risk of community-acquired LOS include poor hygiene, poor cord care, bottle-feeding, and prelacteal feeds. In contrast, breastfeeding helps in prevention of infections**(Wolach,1997 andBaltimore, 1998).**

Signs of sepsis in neonates are often non-specific and high degree of suspicion is needed for early diagnosis. Several laboratory parameters (e.g. complete and differential blood counts, C-reactive protein and blood cultures) can be helpful for screening of neonates with neonatal sepsis **(Stefanovic, 2011).**With early diagnosis and treatment, infants are not likely to experience long-term health problems associated with neonatal sepsis **(Weber *et al*., 2009).**Blood culture is the gold standard for diagnosis of sepsis but blood culture reports are usually available after 48 to 72 hours. There is need to identify the common bacteria causing such infections in every hospital and their susceptibility patterns in order to provide necessary information for timely intervention **(Shresthа*et al*., 2013).**

The bacteria that cause neonatal sepsis are acquired shortly before, during, and after delivery. They can be obtained directly from mother's blood, skin, or vaginal tract before or duringdelivery or from the environment during and after delivery. *Streptococcus agalactiae*(Group B streptococcus, GBS) is the most common cause of neonatal sepsis in many countries, though low rates are reported from many low-income countries, especially those in south Asia **(Zaidi*et al*.,2005 and 2009).**

Gram-negative bacilli (*E.coli*,*Klebsiella* spp., *Pseudomonas*spp*andAcinetobacter* spp.) and gram-positive cocci

(such as *Staphylococcus aureus* and *S.epidermidis*) are other important causes of LONS **(Zaidi*et al*.,2009 and2005)**.

The most common pathogens causing bacterial sepsis and their antibiotic sensitivity patterns vary in different parts of the world. Infections caused by aerobic gram-negative bacilli are common in hospitalized patients resulting in serious infections, and are associated with high mortality rates. This problem has been compounded by the emergence of Gram-negative bacilli that contain extended-spectrum beta- lactamases (ESBLs)**(Fraimow and Tsigrelis, 2011).**

Pathogens causing neonatal infections and their antibiotic susceptibility patterns may change over time and differ between countries **(Anwer*et al.*, 2000; May *et al.*, 2005 and Zaidi*et al.*, 2005).** It is extremely important to diagnose the cases in time so that appropriate antibiotic treatment can be given. Moreover, neonatal infection surveillance networks have been established in several countries and are useful for documenting changes in clinical practice, monitoring changes in pathogens and their antibiotic resistance over time, informing policy and improving quality of care. Thus, the bacterial pathogens responsible and their susceptibility pattern should be regularly monitored in a hospital setting **(Mahmood*et al.*, 2002 and Gray, 2007).**

Antibiotic resistance is now a global problem. Reports of Multi-resistant bacteria causing neonatal infection in developing countries are increasing, particularly in intensive care units**(Fahmey,2013)**.Because of resistance to numerous antimicrobial agents, management and treatment of ESBL-producing *Klebsiella* infections can be challenging and is evolving. It has a high mortality rate of approximately 50% even with antimicrobial therapy.Therefore studying the bacteriologic and antibiotic susceptibility profile of offending pathogens to admitted neonates, can provide a useful guide to the existing pattern of neonatal sepsis. **(Malakan&Momtazmanesh, 2004).**

Empirical combination antibiotics therapy isrecommended for severe sepsis and septic shockcaused by Gram-negative bacteria to reduce mortalityrelated to inappropriate antibiotic treatment.Definitive combination therapy has not been provensuperior to monotherapy with a broad-spectrum beta-lactamfor patients with Gram-negative sepsis but isassociated with an